40509

National Library of Canada / Bibliothèque nationale du Canada

CANADIAN THESES ON MICROFICHE / THÈSES CANADIENNES SUR MICROFICHE

NAME OF AUTHOR/*NOM DE L'AUTEUR* Sara Swenson

TITLE OF THESIS/*TITRE DE LA THÈSE* Combined effects of microwaves and gamma-rays on cultured Mammalian cells

UNIVERSITY/*UNIVERSITÉ* University of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED/ *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* M. Sc

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1979

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. J. W. Scrimger

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED/*DATÉ* April 23, 1979 SIGNED/*SIGNÉ* Sara Swenson

PERMANENT ADDRESS/*RÉSIDENCE FIXÉ* 5475 Carlson Rd
St. Paul, Minnesota
USA 55112

NL-91 (3-74)

National Library of Canada

Cataloguing Branch
Canadian Theses Division

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada

Direction du catalogage
Division des thèses canadiennes

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (3/77)

THE UNIVERSITY OF ALBERTA

COMBINED EFFECTS OF MICROWAVES AND GAMMA-RAYS ON CULTURED MAMMALIAN CELLS

by

© SARA SWENSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

PHYSICS

DEPARTMENT OF PHYSICS

EDMONTON, ALBERTA

SPRING, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Combined effects of microwaves and gamma-rays on cultured mammalian cells," submitted by Sara Swenson in partial fulfilment of the requirements for the degree of Master of Science.

J. W. Scrimger
Supervisor

S. R. Lenton

J. C. Kaleigh

P. D. Thomas

Date April 17, 1979

To my Mother and Father . . .

for their love and encouragement

ABSTRACT

The response of lymphocytic mouse leukemia (L1210) cells to simultaneous microwave and γ-ray exposure has been studied. Specifically, nonthermal microwave effects were investigated. Modification of both growth and survival characteristics were used as indices of cellular response.

An eight kilocurie Cobalt-60 teletherapy unit was used, along with a low intensity RF generator to expose L1210 cells to various combinations of microwave and γ-radiation. Cell suspensions were exposed to microwaves alone, γ-rays alone, and microwave radiation before, after, and during γ-irradiation.

Exposure of L1210 cells to 2450 MHz microwaves with an incident power of 100 mW did not alter the growth characteristics from that of control cell cultures. Cells irradiated to varying doses of γ-radiation showed a decrease in cell survival with increasing dose.

The growth characteristics of L1210 cells exposed to microwaves before, after, or during γ-irradiation did not differ significantly from the growth characteristics of cells which received the same dose of γ-rays.

ACKNOWLEDGEMENTS

I would like to thank all the staff of the Medical Physics Department at the Cross Cancer Institute for their unlimited assistance during my studies and research. I wish especially to thank my supervisor, Dr. J. W. Scrimger, and Dr. S. R. Usiskin, for their aid and advice; and Robin Barnett for always being willing to help in any way possible.

I would like to thank the staff of the McEachern Cancer Research Unit for all their help: Dr. A. R. P. Paterson for supplying me with mouse leukemia cells to carry out my research; and Barb Woima and Milada Selner for patiently instructing me in the art of tissue culture and colony formation.

I want to express my thanks to Dr. Wayne Tinga of the Electrical Engineering Department for kindly providing me with the necessary microwave equipment for my research.

I wish to thank all the staff members at the Cross Cancer Institute who generously gave their time, knowledge, and use of their equipment and facilities.

I would also like to thank everyone involved in the final preparation of this thesis, especially Karl Leisner for his graphic work and photography in Chapter V, and Diana Zaiffdeen for the excellent job she did typing this thesis.

Finally, I would like to thank Shaen for his support, patience, and understanding.

TABLE OF CONTENTS

Chapter		Page
I	INTRODUCTION	1
II	IONIZING RADIATION	9
	2.1 Radiation Physics and Chemistry	9
	2.1.1 Interaction of x-rays and γ-rays with Matter	9
	2.1.2 Physical and Chemical Stages	16
	2.1.3 Radiolysis of Water	23
	2.1.4 Direct and Indirect Action of Radiation	25
	2.1.5 Radiation Protection and Sensitization	29
	2.2 Effect of Ionizing Radiation on Biological Macromolecules	32
	2.2.1 Biological Macromolecules	32
	2.2.2 Proteins	34
	2.2.3 Nucleic Acids	39
III	NON-IONIZING RADIATION	46
	3.1 Microwave Biophysics	46
	3.1.1 Interaction of Microwaves with Biological Systems	46
	(A) Physical characteristics of biological systems	46
	(B) The wave equation in biological media	48
	3.1.2 Microwave Absorption in Biological Systems	53
	(A) General absorption characteristics	53
	(B) Biological dispersions	59

Chapter

Page

3.1.3 Thermal and Nonthermal Microwave Effects . . . 64

(A) Thermal effects 64

(B) Specific thermal effects 67

(C) Nonthermal effects 68

3.1.4 Microwave Effects at the Molecular Level . . . 71

3.2 Effects of Microwaves on Biological Macromolecules . 75

3.2.1 Proteins 75

3.2.2 Nucleic Acids 80

3.3 Whole Body Microwave Absorption 82

IV RADIOBIOLOGY OF CULTURED MAMMALIAN CELLS 91

4.1 Cells in Culture 91

4.1.1 The Cell Cycle and Growth Cycle 92

4.1.2 Growth Curves 94

4.1.3 Cell Death 95

4.1.4 Target Theory and Survival Curves 97

4.2 Effect of Ionizing Radiation on Cells 100

4.3 Effect of Microwaves on Cells 106

4.4 Combined Effects of Ionizing Radiation and Microwaves on Cells 111

V EXPERIMENTS 114

5.1 Methods and Materials 114

5.1.1 Routine Procedures 114

5.1.2 Irradiation of Cell Suspensions with γ-Rays . 118

(A) Growth curves 118

(B) Survival curves 119

Chapter Page

5.1.3 Exposure of Cell Suspensions to 2450 MHz Microwaves 121

(A) Microwave apparatus and dosimetry 121

(B) Experimental procedure 125

5.1.4 Combined Microwave and γ-Ray Irradiation . . . 127

5.2 Results 130

5.2.1 Growth Curves under Varying Experimental Conditions 130

5.2.2 Growth Curves of γ-Irradiated Cultures 135

5.2.3 Growth Curves of Cells Exposed to Microwaves and Hyperthermia 135

5.2.4 Growth Curves of Combinations of γ-Ray and Microwave Exposures 139

5.2.5 Survival Curves 145

VI CONCLUSION AND DISCUSSION 150

BIBLIOGRAPHY 154

APPENDIX I 173

LIST OF TABLES

Table		Page
I	The electromagnetic spectrum	3
II	Radiation chemical reactions	21
III	Reactions of e^-_{aq}, ·OH, and H·	26
IV	Product yield of various radicals and molecules in water for low LET radiation	28
V	Characteristics of electromagnetic wave propagation in tissues of high water content	57
VI	Characteristics of electromagnetic wave propagation in tissues of low water content	58
VII	Activation energy and radiation parameters for various molecular microwave effects in biological systems	73
VIII	Reported microwave and radiofrequency effects in biological systems	84
IX	Culture medium composition	115

LIST OF FIGURES

Figure		Page
2.1	X-ray and γ-ray interactions in biological systems	12
2.2	Ionization pattern caused by an energetic primary electron passing through water	14
2.3	Approximate time scale in radiation chemistry	17
2.4	Potential energy curves for a diatomic molecule in the ground state and first excited state	19
2.5	Electronic energy levels	19
2.6	Structure of an amino acid	35
2.7	DNA structure	41
3.1	Relationship between the electric and magnetic field in an electromagnetic wave	52
3.2	Variation of K_e and σ with frequency	61
3.3	Dispersions for human muscle tissue	61
3.4	Orientation of a dipole with an electric field	66
3.5	The pearl chain effect	69
3.6	Microwave interactions with biological systems	76
4.1	Growth curve for L1210 cells	96
4.2	Survival curve for L1210 cells	98
5.1	Microwave apparatus	123
5.2	Theratron 80 and microwave apparatus	128
5.3	Control growth curves	131
5.4	Control growth curves after irradiation with γ-rays . . .	133

Figure		Page
5.5	Growth curves after γ-irradiation under a reduced atmospheric oxygen concentration	133
5.6	Growth curves after γ-irradiation and incubation at 39° C	134
5.7	Growth curves after γ-irradiation under a reduced atmospheric oxygen concentration and incubation at 39° C	134
5.8	Growth curves after γ-irradiation	136
5.9	Growth curves after exposure to 2450 MHz microwaves . . .	137
5.10	Growth curve after 30 minutes microwave exposure	137
5.11	Growth curves for 15 minute exposures to heat and microwaves	140
5.12	Growth curves for 30 minute exposures to heat and microwaves	140
5.13	Growth curves for 45 minute exposures to heat and microwaves	141
5.14	Growth curves for 60 minute exposures to heat and microwaves	141
5.15	Growth curves for simultaneous γ-ray and microwave exposure (#1)	143
5.16	Growth curves for simultaneous γ-ray and microwave exposure (#2)	143
5.17	Growth curves for microwave exposure before and after 500 rads of γ-radiation	144
5.18	Microwave exposure before and after 250 rads of γ-radiation	144

Figure		Page
5.19	Plating efficiency for colony-forming ability	147
5.20	Survival curve for simultaneous microwave and γ-ray exposure compared with γ-ray survival	148

CHAPTER I

INTRODUCTION

As early as 1000 B.C., there have been reports concerning the biological effects of electromagnetic waves. The early reports centered mostly upon the effects of sunlight on human skin. It was not until 1865 that Maxwell determined that the velocity of propagation of electromagnetic waves was equal to that of visible light. This meant that visible light was electromagnetic radiation, and that it was the first type of electromagnetic radiation known to cause a biological response. Maxwell's discovery led eventually to the identification of all parts of the electromagnetic spectrum.

By the beginning of the 1900's, other components of the electromagnetic spectrum had been identified. In 1888, Hertz experimentally produced radiofrequency electromagnetic radiation. The beginning of radiation biology occurred in 1895 with the discovery of x-rays by Roentgen, and the fact that they could penetrate human skin. A few years later, the Curies and Bequerel discovered the characteristics of γ-rays emitted by radioisotopes. In fact, in 1902 the earliest known case of radiation-induced cancer was reported. Since that time, many sources of ionizing radiation have been identified and the amount of radiation delivered to an organism from each source can be quantified. It is now possible to determine the relationship between exposure to ionizing radiation and the absorbed dose in an organism, and the subsequent biological response (Hine and Brownell, 1956; Bacq and Alexander, 1961;

Attix, Roesch, and Tochilin, 1968).

The electromagnetic spectrum consists of all electromagnetic radiation ranging from high energy x-rays to low energy radiowaves. The electromagnetic spectrum is arbitrarily divided into regions and given a spectral designation as shown in Table I. There are two broad spectral classifications based on photon interactions with living matter; high energy or "ionizing" radiation, and low energy or "non-ionizing" radiation.

The high energy portion of the electromagnetic spectrum, with the shortest wavelengths, highest frequencies, and energy greater than 10 eV, causes ionizations in living matter. It consists of γ-rays, x-rays, and far ultraviolet.

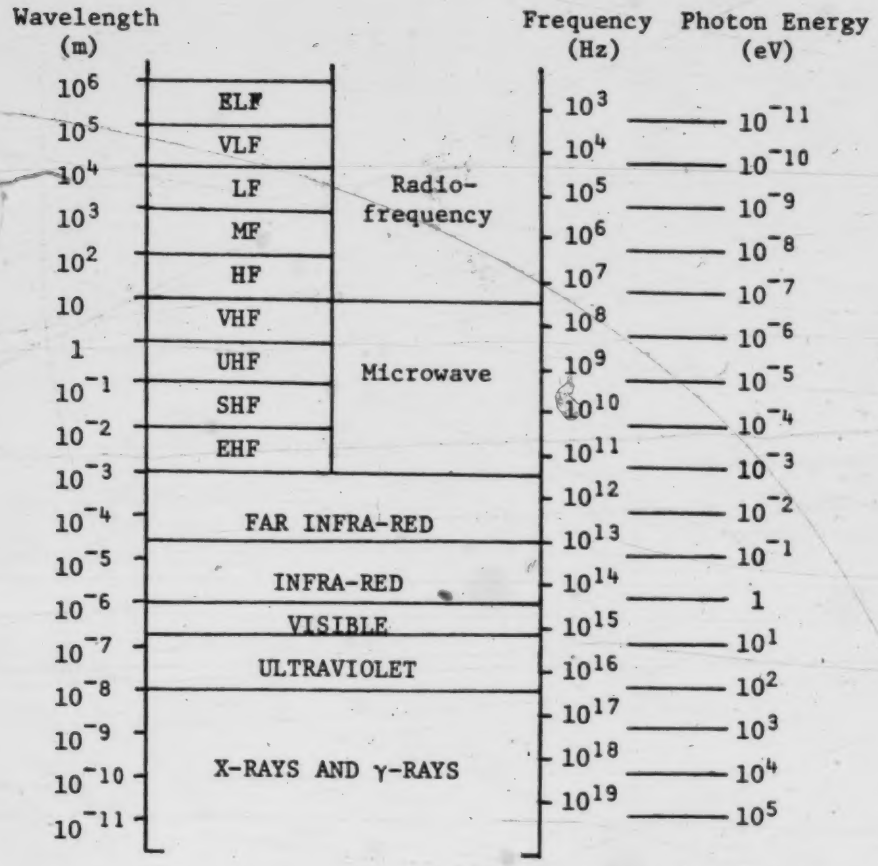
The part of the electromagnetic spectrum which does not cause ionizations in living tissue, with energy less than 10 eV, consists of near ultraviolet, visible light, infrared, microwaves, and radiofrequency waves, and is called "non-ionizing" radiation. Microwave radiation lies between the frequencies 30 MHz and 3×10^5 MHz, corresponding to wavelengths in air of ten meters to one millimeter. Radiofrequency waves have a frequency that is less than 30 MHz, and a corresponding wavelength in air greater than ten meters. The radiofrequency portion of the spectrum can be further divided into short, median, long, and very long waves. The designation used for radiofrequency waves and microwaves is shown in Table I.

Until about 1930, most research on the biological effects of electromagnetic radiation had dealt with ionizing radiation; that radiation with gross biological effects. However during the decade

TABLE I

THE ELECTROMAGNETIC SPECTRUM

(Kaye and Laby, 1973)



SYMBOLS

LF = low frequency

HF = high frequency

MF = medium frequency

E = extra

V = very

U = ultra

S = super

1930-1940, interest in the biological effects of non-ionizing radiation began to increase (Carpenter and Page, 1930; Krusen et al., 1947; Michaelson, 1972). The first investigations centered on the molecular and chemical effects with a resultant biological response in elementary biological systems (Kahler et al., 1929; Pereira, 1935; Fleming, 1944; Nyrop, 1946). After World War II, interest switched to whole-body microwave effects in mammals and man (Clark, 1950; Kalant, 1959; Dodge, 1970; Healer, 1970; Cleary, 1970). In some cases, people were accidentally exposed to a high enough intensity of microwaves to cause a detectable heating sensation in the skin (Daily, 1943; Cook, 1952; Hendler et al., 1963; Michaelson et al., 1964; Hendler, 1968). This microwave effect, hyperthermia, has been extensively studied in the last few decades (Krusen et al., 1947; Schwan and Piersol, 1954, 1955; Har-Kedar and Bleehan, 1976; Bronk, 1976; Mendecki et al., 1978). Mechanisms for this effect have been postulated and have been well documented (Schwan et al., 1966; Cleary, 1970; Stuchly and Hamid, 1972; Baranski and Czerski, 1976).

While much has been learned about hyperthermia and how it is caused in animals and man by microwave energy absorption, not all biological effects caused by microwaves can be attributed to hyperthermia (Presman, 1965, 1970; Cleary, 1973, 1977). There are, in fact, a growing number of biological effects which may be caused in vivo or in vitro by low intensity microwaves (Heller and Teixeira-Pinto, 1959; Teixeira-Pinto et al., 1960; Sher, 1970; Presman, 1970; Michaelson, 1971; Michaelson and Dodge, 1971; Barnes and Hu, 1977; Cleary, 1977). These include central nervous system effects (Presman, 1965), reversible effects (Presman, 1970), specific sensory effects (auditory and olfactory) (Frey, 1962;

Michaelson, 1972), cardiovascular effects (Presman, 1965), pearl chain formation (Herrick, 1958; Heller, 1959; Saito and Schwan, 1961; Johnson and Guy, 1972), resonance absorption by cells and molecules (Vogelhut, 1960; Prausnitz et al., 1961), dielectric saturation (Schwan, 1958), and dielectric dispersion of cells and biomolecules (Schwan, 1957, 1958, 1974). These effects are manifested without the occurrence of a detectable increase in temperature. In contrast to high intensity microwaves which produce thermal effects by increasing the average kinetic energy of molecules in a system, athermal effects are defined as being due to an interaction of the incident electromagnetic fields with specific receptor molecules (Cleary, 1970). Energy absorbed by a receptor would remain there for a period of time causing some kind of functional alteration before being passed on to other molecules as thermal energy.

Many athermal effects are manifested at a low field intensity of long duration. The question arises at what intensity can athermal damage be induced? At present, it is known that exposure to microwave radiation in the gigahertz frequency range at intensities of 10 mW/cm^2 or more for periods of an hour or longer will cause a detectable increase in core temperature (Cleary, 1970). This sets the arbitrary boundary between high and low intensity microwaves. Microwave power densities of 10 mW/cm^2 for periods of more than a few minutes are considered as high intensity exposures, whereas low intensity exposures are those less than 10 mW/cm^2.

Determining if athermal effects occur by a different mechanism than thermal effects and quantitating the absorbed dose necessary for an athermal effect to occur is difficult (Johnson and Guy, 1972; Johnson, 1973). As well, the threshhold of intensity and duration for manifestation of an athermal effect is not yet known (Presman, 1970). Since an

athermal microwave effect cannot be determined by a temperature increase as can hyperthermic effects, it is necessary to devise some means of determining whether exposure to low intensity microwaves causes a biological response and to define the basic mechanisms of that response (Illinger, 1974; Schwan, 1974).

For many years, it has been known that hyperthermia induced in mammalian cancer cells sensitized the tumour cells to ionizing radiation (Cavaliere et al., 1967; Mondovi et al., 1969; Ben-Hur et al., 1974, 1978; Gerweck et al., 1975; Harisiadis et al., 1978; Streffer et al., 1978). The combined effect of microwave-induced hyperthermia and ionizing radiation on mammalian cells is greater than that of either type of radiation used alone. This is referred to as a synergistic effect. The question arises whether synergistic effects may also occur through the use of combinations of low intensity microwaves and ionizing radiation. If a synergistic effect does occur, then it may be used to determine if low intensity microwaves affect mammalian cells when it is not possible to directly determine whether low intensity microwaves evoke a biological response.

The first step is to determine whether a synergistic effect occurs in mammalian cells in vitro. This may lead to the determination of the mechanism of the effect, if any occurs. Even though cell and tissue cultures provide a valuable technique for studying the effects of various chemical and physical agents on biological systems, it is not always possible to transfer the results to in vivo situations. Cells in tissues in an organism often function differently from cells suspended in an aqueous medium, which may have become dedifferentiated. However, the use

of cell cultures may lead to determination of the mechanism by which an effect occurs.

The use of cellular growth and survival curves before and after introduction of a chemical or physical agent to a mammalian cell culture will give a good indication of the extent of lethality of the agent to the cells in suspension (Elkind and Whitmore, 1967). In this project, growth and survival curves will be determined for a mammalian cell line exposed to microwaves alone, γ-rays alone, and combinations of microwaves and γ-rays. It is known that exposure to γ-rays causes cell killing. At present, it is difficult to detect the effects of low intensity microwaves at the cellular level because they are not lethal, but they may alter cellular structure or function in some manner. If no detectable alteration of a normal growth curve occurs after exposure of the cells to low intensity microwaves alone, then a combination of γ-ray and microwave exposure may be used to determine if a microwave effect occurs. If a survival curve of mammalian cells in vitro which are exposed to both microwaves and γ-rays differs from a survival curve of cells exposed to γ-rays alone, then it can be postulated that microwaves perturb the cellular environment. This may be either a sensitizing or protective effect. If the combined effect is greater than the sum of both microwaves and γ-rays alone, then it is referred to as a synergistic effect. If no combined effect occurs, then it can be concluded that low intensity microwaves do not perturb the cellular environment to a sufficient degree to increase the number of sublethal or lethal events produced by γ-rays.

Today, there is an increasing use of instruments which produce

non-ionizing radiation either directly or indirectly. Radiofrequency waves are used extensively for communication systems, whereas microwaves are used in communications, radar, diathermy in medicine (Schwan, 1965; Lehman, 1976), in industry, and in the home (Michaelson, 1972). With this continually increasing production of microwaves and radiofrequency waves, there exists a need to determine the biological consequences of non-ionizing radiation (for references, see Glaser, 1971-1975), its method of interaction with matter, and to be able to correlate exposure with absorbed dose in biological systems. In essence, with such a large volume of knowledge still to be gained on the biological effects of microwaves, this project will try to explore one aspect of microwave interaction with a biological system. The presence of thermal effects has been well established and methods of dosimetry have been studied. However, very little is known about the nature of athermal microwave effects. This project will attempt to determine whether low intensity microwaves and γ-rays exert a synergistic effect on a mammalian cell line, specifically mouse lymphoma (L1210) cells. This indirect method of determining whether microwaves affect the cellular environment is used in order to overcome the difficulty of measuring sublethal cellular events. If any enhancement in either cell growth or cell killing occurs, then the conclusion can be drawn that athermal microwave effects exist. However, if no enhancement occurs, the possibility still exists that athermal cellular effects are a function of microwave parameters such as intensity, frequency, mode, and duration, or a function of the biological system. It will then be necessary to devise another means of exploring the manifestations of cellular athermal microwave effects.

CHAPTER II

IONIZING RADIATION

2.1 Radiation Physics and Chemistry

2.1.1 Interaction of x-Rays and γ-Rays with Matter

X-rays and γ-rays are ionizing electromagnetic radiation and differ only in their origin.

X-rays are produced when fast electrons are decelerated in materials with a high atomic number. When incident electrons interact with the nuclear field of target atoms, a continuous spectrum of x-rays is produced. The upper limit of this spectrum is determined by the maximum kinetic energy of the incident electrons. Characteristic x-rays are emitted in atomic transitions of bound electrons between the shells of atoms. When a fast electron displaces an orbital electron from the inner shell of a target atom, an outer electron takes its place, and an x-ray is emitted provided the transition is not forbidden by quantum restrictions. The energy of the x-ray is determined by the difference in energies between the inner and outer shell, and thus is characteristic for the target element.

γ-rays originate in the nucleus and accompany nuclear transitions. They are produced when an unstable nucleus releases energy during transition to a more stable state.

"The exposure, X, is the quotient of dQ by dm where dQ is the absolute value of the total charge of the ions of one sign produced in air

when all the electrons (negatrons and positrons) liberated by photons in a volume element of air having mass dm are completely stopped in air." (ICRU 19)

$$X = \frac{dQ}{dm}$$

The special unit of exposure is the roentgen (R) where 1 R = 2.58×10^{-4} Coulomb/kilogram.

The absorbed dose of x-rays or γ-rays in a material is the amount of energy absorbed per unit mass of irradiated material. It is a direct measure of the energy transferred to the medium by the radiation which can cause physical or chemical change in the irradiated medium. Absorbed dose is measured in grays or rads. One gray is defined as an energy absorption of 1 J/kg (ICRU 19). One rad is equal to 1/100 of a gray absorbed dose.

A beam of x-rays or γ-rays, incident on a target material, will penetrate the material and interact with atomic and molecular electrons causing a transfer of energy to the medium. As photons are removed from the beam (by transfer of energy to the material) the intensity decreases, and the beam is attenuated.

The attenuation of x-rays and γ-rays in a material for a narrow monoenergetic beam is exponential and is given by the relationship:

$$\frac{I}{I_o} = e^{-\mu d}$$

where $\frac{I}{I_o}$ = fraction of photons remaining in the beam after passage through an absorber of thickness d

μ = total linear attenuation coefficient

For a broad beam of photons, a "build-up" factor must be added to the right hand side of the above equation to account for scattered radiation into and out of the path to the detector. This is given by:

$$\frac{I}{I_o} = Be^{-\mu d}$$

The build-up factor is the ratio of transmitted intensity, which includes attenuated primary radiation plus scattered radiation entering the detector, to the intensity of the attenuated primary radiation only.

X-rays and γ-rays interact with matter primarily by three mechanisms: photoelectric absorption, Compton scattering, and pair production (Figure 2.1). The linear attenuation coefficient (μ) is the sum of the cross sections for the three effects, each of which depends on photon energy and the atomic number of the absorber.

Photoelectric absorption occurs when a low energy photon of less than 100 keV is absorbed by an atom. This results in an ejected photoelectron with kinetic energy equal to the difference between the incident photon energy and the binding energy of the electron. The atomic cross section ($_a\tau$) is proportional to $Z^4/(h\nu)^{7/2}$ (Heitler, 1954; Evans, 1955).

Compton scattering predominates for tissue-equivalent materials at photon energies of 0.1-1.0 MeV. In this case, a photon interacts with a "free" electron, and the photon is deflected with a longer wavelength. Because the photon energies in this range are much greater than the orbital energies, electrons are scattered as free particles. Both the scattered photon and the recoil electron may cause further ionizations. The electronic cross section for Compton attenuation is independent of Z because the electron density is approximately the same

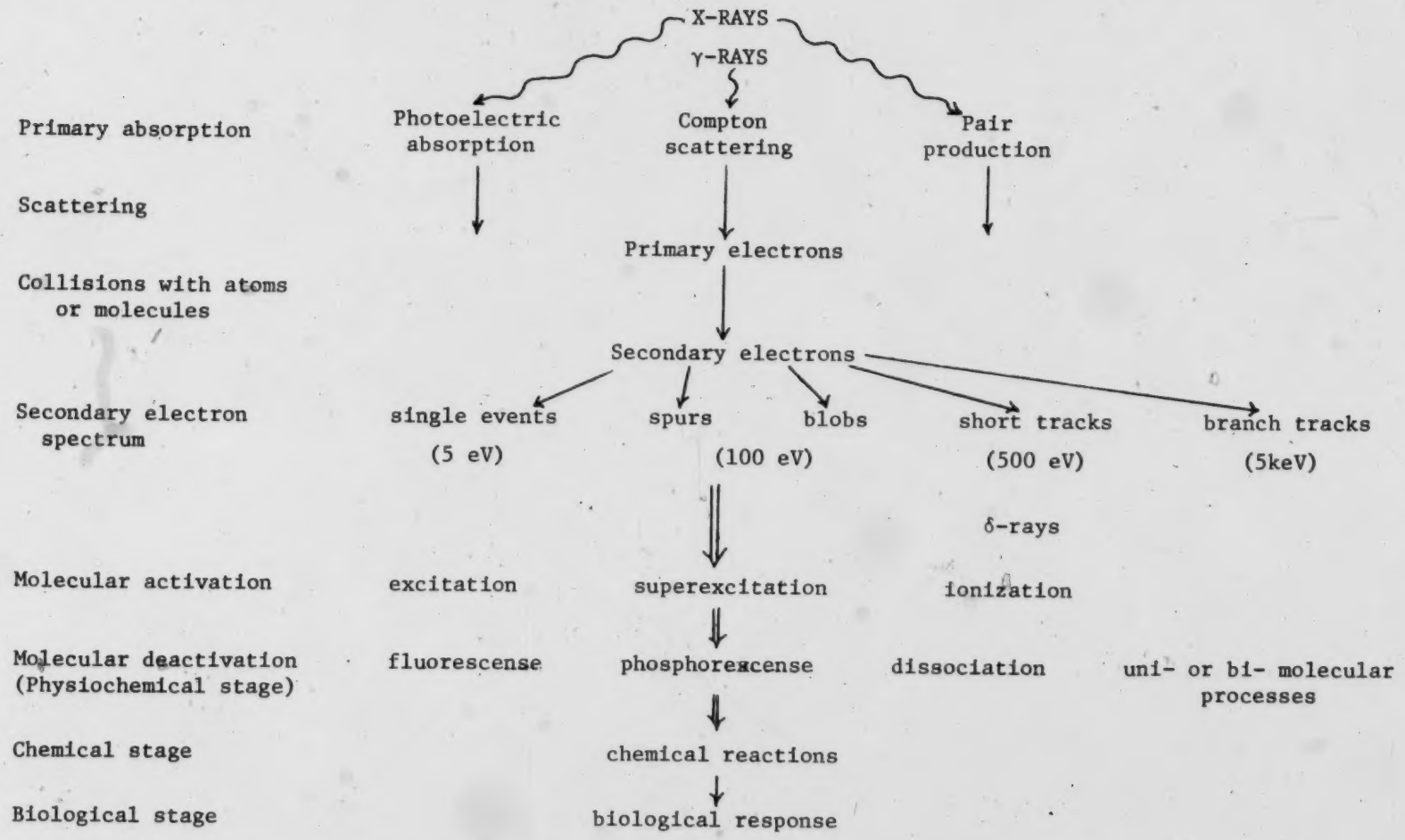


Fig. 2.1 X-ray and γ-ray interactions in biological systems.

for all solids and liquids (Evans, 1955).

Pair production becomes possible at photon energies greater than 1.02 MeV (equivalent to the rest mass of the positron and electron). An electron-positron pair is generated when a high energy photon interacts with the field of a nucleus. If the photon has an energy greater than 1.02 MeV, the excess energy will be distributed as kinetic energy between the positron and electron. The energy will be transferred to the medium through subsequent excitations and ionizations. The pair production cross section per atom ($_a\kappa$) varies approximately as Z^2 (Heitler, 1954; Hine and Brownell, 1956).

Photon interactions with matter by these three processes give rise to electrons with a range of energies which travel through the medium. Energy is lost by the primary electrons when they are slowed down through collisions with molecular electrons. This gives rise to excited and ionized molecules along the path of the primary electron. The primary electrons may produce secondary electrons with a range of energies which may also cause excitations and ionizations. Mozumder and Magee (1966) have classified these secondary or later generation electrons according to their energy and the ionization patterns they produce (Figure 2.2).

A glancing collision between an electron and molecule will result in an energy transfer to the molecule of between 9 and 100 eV in an isolated event. This may cause an excitation or several ionizations of molecular electrons. The range of these secondary electrons will be short and the track of ionizations they produce will be close to the track of the original ionizations. This is referred to as a spur. Most spurs contain one to four ionization events and several excited molecules.

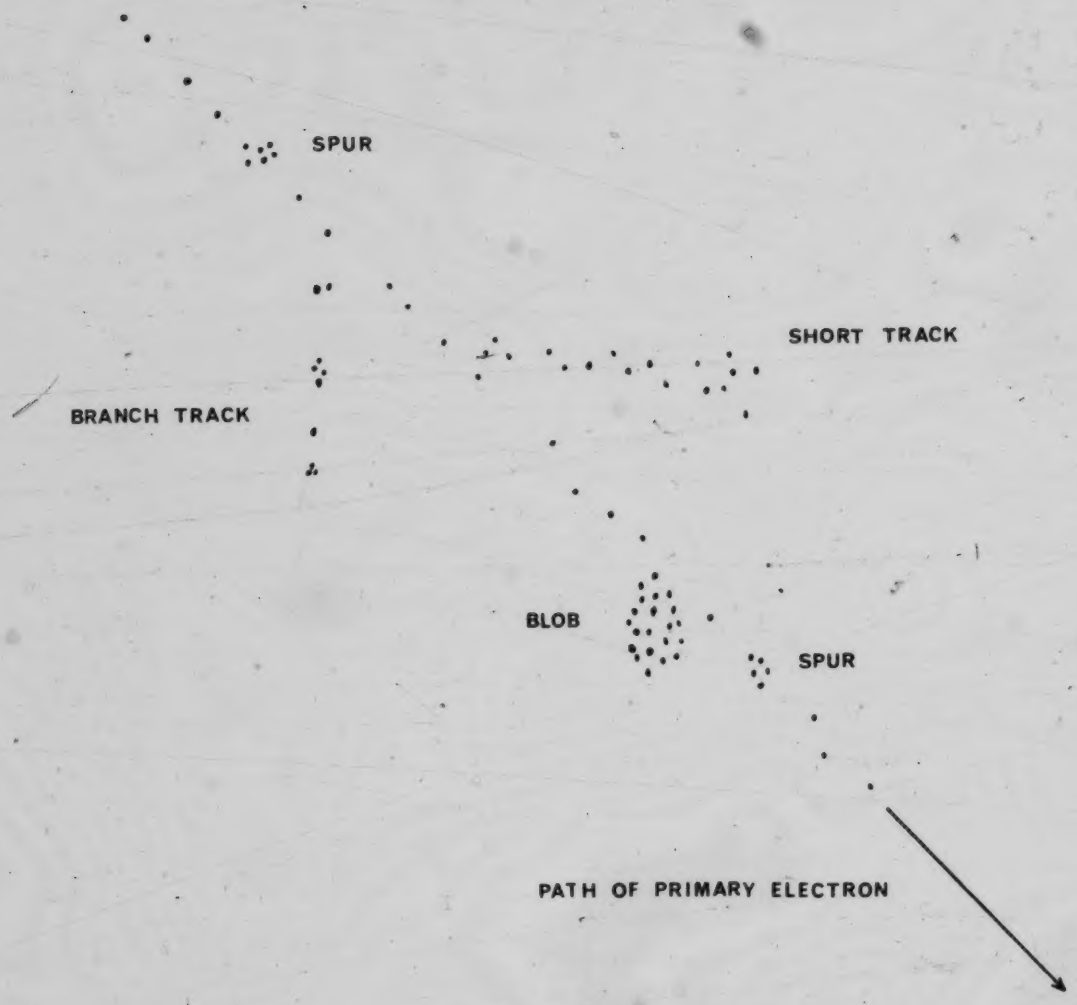


Fig. 2.2 Ionization pattern caused by an energetic primary electron passing through water.

A knock-on collision between an electron and molecule will produce a track of fast secondary electrons or δ-rays. When 100-500 eV is transferred to the secondary electron, the path of ionizations looks like a large spur and is referred to as a blob. A blob occurs because the secondary electrons do not have enough energy to leave their site of origin. A short track arises from an energy transfer of 500-5000 eV and has overlapping spurs. This distinguishes it from a branch track which is produced by a secondary electron with kinetic energy greater than 5 KeV and does not have overlapping spurs.

If an aqueous solution is being irradiated by Cobalt-60 γ-rays, the high energy photons interact with the water molecules to produce mainly Compton electrons with a wide range of energies (10^2-10^6 eV). The LET of the high energy electron is low and generally will produce depositions of energy of about 100 eV. This causes formation of a spherically symmetric spur. These spurs are usually far enough apart so that they will not interact with each other. Low energy electrons produce spurs which overlap. The primary reactive species from overlapping spurs may interact (Kupperman, 1967). Thus, for Cobalt-60 γ-rays, there is an LET distribution which causes production of overlapping spurs as well as the predominant isolated spherical spurs.

To compare the effects of various types of radiation, the linear energy transfer (LET) expressed in KeV/micron is used. This is the average energy released per unit track length and includes both excitations and ionizations. The LET "L_Δ, of charged particles in a medium is the quotient of dE by dl, where dl is the distance traversed by the particle and dE is the mean energy-loss due to collisions with energy

transfers less than some specified value Δ." (ICRU 16)

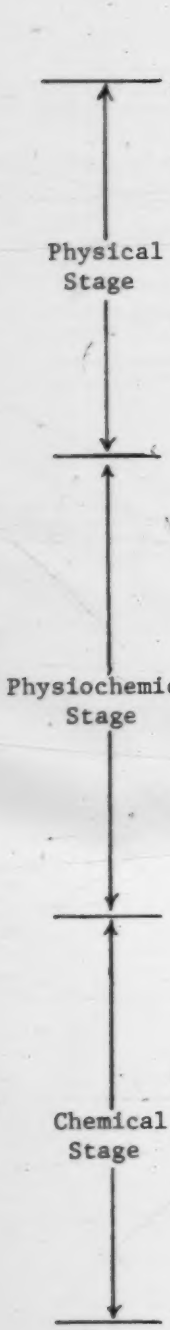
$$L_\Delta = \left(\frac{dE}{dl}\right)_\Delta$$

A high LET is produced by a slow electron which deposits all its energy in a cluster, causing a densely ionized track. A fast electron has a low LET and produces a track with evenly spaced spurs. Thus, the distribution of ionizations and excitations is a function of the LET of the particle, which depends on the energy.

2.1.2 Physical and Chemical Stages

The absorption in matter of ionizing radiation can be subdivided into three or four consecutive stages (Figure 2.3). The physical stage involves the dissipation of the absorbed energy in the system by the formation of a large number of activated, unstable molecules. The duration of this stage is less than 10^{-13} second. In the physicochemical stage, of the order of 10^{-11} second or less, the unstable molecules undergo secondary reactions and cause the system to reach thermal equilibrium. During the chemical stage, the newly formed reactive species, such as ions or free radicals, interact with each other or the medium. This occurs from 10^{-9} to 10^{-6} second and leads to the establishment of chemical equilibrium in the system. In a biological system, a fourth or biological stage occurs. This involves the response of the system to the chemical changes and products which were formed during the irradiation. Biological responses occur on a very different time scale and may be manifested any time after the chemical stage.

The physical stage is characterized by the transfer of energy from



Time (sec)	Event
10^{-18}	Electron with energy in the MeV range traverses a molecule
10^{-16}	Loss of energy by secondary electrons
10^{-15}	Time between successive ionizations produced by an MeV electron
	Time for "vertical" excitation to an electronic excited state
10^{-14}	Ion-molecule reactions
	Period of molecular vibrations
10^{-13}	Secondary electrons reduced to thermal energy (about 0.025 eV)
10^{-12}	Radical moves one molecular diameter by diffusion
10^{-11}	Relaxation time for the dipole orientation of water molecules
	Ions and electrons become solvated
10^{-10}	Minimum time for diffusion-controlled reactions in the spur
10^{-8}	Formation of molecular products complete in the spur
10^{-5}	Reactions between radicals and solute
10^{-4}	Radical diffuses the inter-spur distance in the track of an MeV energy electron
1	Chemical reactions complete

Fig. 2.3 Approximate time scale in the radiation chemistry of an aqueous solution (Spinks and Woods, 1976).

the incident photons to the molecular structure of the material causing production of energetic electrons. These electrons, in turn, transfer energy to the electronic system of the molecules in the material causing molecular activation, such as excitation, superexcitation, and ionization. Ionization occurs when the energy imparted to an electron is sufficient to eject an electron from its orbital. If the energy transferred to an atom or molecule is only enough to raise an electron to a higher energy level, the molecule exists in an excited state. When a molecule has been excited to a level above its ionization potential, it may have a transient existence in this superexcited state or it may ionize in the process. The molecule may then lose energy through dissociation or by emitting an electron.

The primary activation of molecules takes place in a time approximately equal to that of the electronic oscillation period of the molecule, or 10^{-15} to 10^{-16} second. Since the period for molecular vibration is 10^{-13} to 10^{-14} second, the nuclei in the molecule are practically stationary during the excitation process. Thus the internuclear distance remains the same and the transition can be represented by a vertical line (O-P) on a potential energy diagram (Figure 2.4). This corresponds to the most probable transition and is known as the Franck-Condon principle. Since the bond vibration frequency is about 10^{13} cycles/sec, molecules excited above their dissociation limit will dissociate in less than 10^{-13} second.

Once molecular excited states have been formed, they may return to the ground state by various unimolecular or bimolecular routes. These transitions occur during the physiochemical stage and enable the system to reach thermal equilibrium.

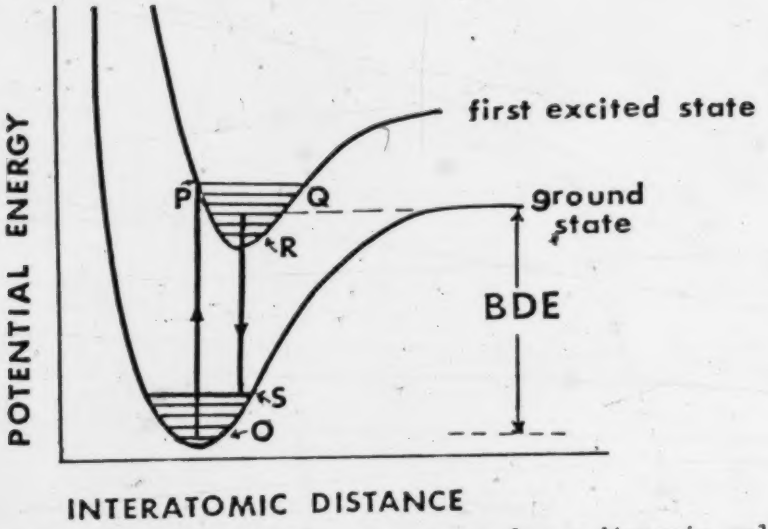


Fig. 2.4 Potential energy curves for a diatomic molecule in the ground state and first excited state. The bond dissociation energy (BDE) is indicated.

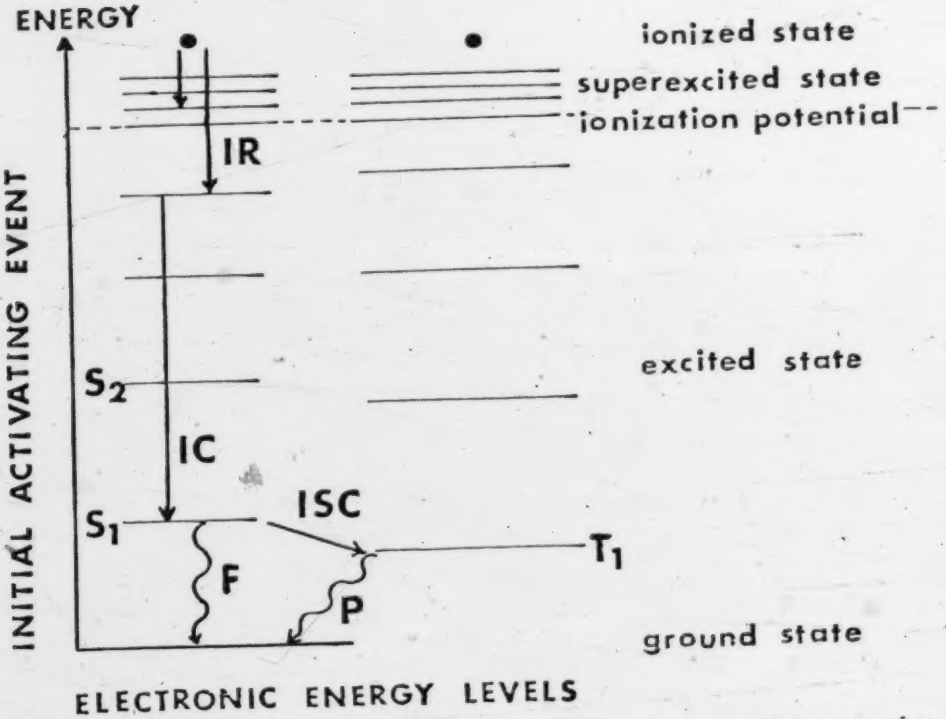


Fig. 2.5 Electronic energy levels: ISC = intersystem crossing; S = singlet state; T = triplet state; IC = internal conversion; IR = ion recombination; F = fluorescence; and P = phosphorescence.

During the physiochemical stage, the excess vibrational energy gained by a molecule is usually lost by collisions with other molecules. This occurs in 10^{-13} to 10^{-12} second. Figure 2.4 shows a molecule dropping from the vibrationally excited state PQ to lower vibrational levels and eventually to R.

An electronically excited molecule may return to the ground state by several different methods (Figure 2.5). These include: fluorescence or radiative conversion to the ground state in 10^{-8} second; internal conversion or nonradiative conversion to the lowest excited state in 10^{-13} second, and then fluorescence to the ground state; intersystem crossing or nonradiative conversion to a state of different multiplicity (i.e., singlet to triplet) and then phosphorescence in 10^{-4} to 10^{-3} second; and nonradiative energy transfer to a neighboring molecule in 10^{-13} second. The most common unimolecular processes undergone by excited states result in dissociation of the molecule into two fragments which may be free radicals (a molecular fragment with an unpaired electron) or stable molecules. This occurs in about 10^{-13} second.

Excited molecules may also interact with a second molecule to cause recombination of fragments or to form new products. These are divided into four categories of reactions: electron transfer, abstraction, addition, and Stern-Volmer reactions (Table II). Since an excited molecule has a higher electron affinity and a lower ionization potential, electron transfer reactions with other molecules may be either reductive or oxidative (Dainton, 1967).

Rotational processes, such as dipole reorientation, occur in a period of the order of the rotational relaxation time of the liquid, about 10^{-11} second in water. This leads to the production of hydrated electrons

TABLE II

Radiation Chemical Reactions
(Spinks and Woods, 1976)

A. Reactions of Excited Molecules

1. Non-radiative energy transfer
   $A^* + B \rightarrow A + B^*$

2. Dissociation into free radicals
   $A^* \rightarrow R^\cdot + S^\cdot$

3. Dissociation into molecular products
   $A^* \rightarrow M + N$

4. Electron transfer
   $A^* + B \rightarrow A^+ + B^-$

5. Hydrogen abstraction
   $A^* + RH \rightarrow AH^\cdot + R^\cdot$

6. Addition
   $A^* + B \rightarrow AB$

7. Stern-Volmer; interchange of atoms between two molecules
   $A^* + B \rightarrow P + Q$

B. Reactions of Ions

1. Neutralization by a negative ion
   $A^+ + A^- \rightarrow A^* + A$

2. Neutralization producing singlet and triplet excited states
   $A^+ + e^- \rightarrow A^{**} \rightarrow A^*$

3. Dissociation into molecular products following neutralization
   A^* (or A^{**}) $\rightarrow M^* + N$

4. Dissociation into free radicals following neutralization
   A^* (or A^{**}) $\rightarrow R^{\cdot *} + S^\cdot$

5. Charge transfer
   $A^+ + B \rightarrow A + B^+$

6. Ion-molecule reactions
   $A^+ + B \rightarrow C^+ + D$

7. Electron capture
   $A + e^- \rightarrow A^-$ or $B^- + C$

Table II (Continued)

C. Reactions of Free Radicals

1.	Dissociation	$AB^{\bullet} \rightarrow A^{\bullet} + B$
2.	Rearrangement	$AB^{\bullet} \rightarrow BA^{\bullet}$
3.	Addition	$R^{\bullet} + C{=}C \rightarrow R{-}C{-}C^{\bullet}$
4.	Oxygen addition	$R^{\bullet} + O_2 \rightarrow R{-}O{-}O^{\bullet}$
5.	Abstraction	$A^{\bullet} + BC \rightarrow AB + C^{\bullet}$
6.	Combination	$R^{\bullet} + S^{\bullet} \rightarrow RS$
7.	Electron transfer	$M^{z+} + R^{\bullet} \rightarrow M^{(z+1)+} + R^{-}$

(e^-_{aq}) (Boag, 1967).

After the duration of the physiochemical stage, about 10^{-11} second, there are many reactive species present which are in thermal equilibrium with the surrounding medium. The reactive species have a specific spatial distribution which is dependent on the quality of radiation used. The radii of spurs formed along the particle track of a fast electron is approximately 10-25 Å. The concentration of the species (radicals and ions) in the spur decreases in time due to reaction of intermediates causing expansion of the spur's effective volume. The expansion of spurs by diffusion and reactions inside the spur occur in about 10^{-11} second. Reactions between reactive species in the bulk medium with each other or with molecules in the medium will occur in approximately 10^{-8} second. The reactions which occur during the chemical stage are shown in Table II. Generally, these reactions are fast, occurring in less than one second, and are mainly diffusion-controlled.

2.1.3 Radiolysis of Water

Since most biological systems are composed of a large percentage of water, the reactions which occur in water and other aqueous solutions are of significant interest. In water, the ionizing radiation causes molecular activation, such as excitation, superexcitation, and ionization. Excited molecules formed directly by the radiation will either return to the ground state by a nonradiative process or dissociate into H· and ·OH radicals. These H· and ·OH radicals are confined by water molecules and since they have little energy, will tend to recombine to form water molecules producing little chemical change. The reaction is given by:

$$H_2O^* \rightarrow \cdot OH + H\cdot \rightarrow H_2O$$

The excited molecules may also combine with each other to form molecular hydrogen and hydrogen peroxide as shown by:

$$H_2O^* + H_2O^* \rightarrow H_2 + H_2O_2$$

The ions which are formed as a result of the irradiation are H_2O^+, H_3O^+, OH^+ and H^+. No H_2O^- is found and only small amounts of OH^- occur. The ions OH^+ and H^+ may be caged by the solvent and may recombine with H· and ·OH respectively to give H_2O^+ before being neutralized as shown:

$$H^+ + \cdot OH \rightarrow H_2O^+$$

$$OH^+ + H\cdot \rightarrow H_2O^+$$

These reactions occur before the ions become solvated.

Ions formed in water will become hydrated if their lifetimes are greater than 10^{-11} second. An ion will orient surrounding water molecules so that the part of the molecule which has a charge opposite to that of the ion will be closest to the ion. This is a state of minimum potential energy for the ion and water molecules. The hydrated ion will be surrounded by a shell of water molecules loosely bound by the electrostatic attraction between the ion and dipole water molecules. Hydrated ions found after irradiation of water by x-rays or γ-rays are H^+_{aq} (H_3O^+) and e^-_{aq}. Hydrated electrons may interact with hydrogen ions to form hydrogen radicals: $e^-_{aq} + H^+ \rightarrow H\cdot$.

The primary reactive species formed after irradiation of water are hydroxyl radicals (·OH), hydrated electrons (e^-_{aq}), and hydrogen atoms (H).

$$H_2O \rightarrow H_2O^+ + e^-$$

$$e^- + (H_2O)_n \rightarrow e^-_{aq}$$

$$H_2O^+ + H_2O \rightarrow \cdot OH + H_3O^+$$

$$H_2O \rightarrow H_2O^*$$

$$H_2O + H_2O^* \rightarrow 2\cdot OH + 2H\cdot$$

$$e^- + H_2O \rightarrow H\cdot + OH^-$$

$$e^- + H_2O^+ \rightarrow H\cdot + \cdot OH$$

These species are initially formed in or near spurs spaced along the particle track in a similar manner to the original ions and excited molecules. The density of the surrounding medium will tend to restrict the radicals and ions to the particle tracks where they will react with each other before the products are diffused throughout the liquid. Reactions in the spur are usually complete in 10^{-10} second. Combination of radicals in the spurs produces molecular hydrogen, hydrogen peroxide, and water. In the case of low LET radiation, such as x-rays or γ-rays, most of the radicals will escape from the spurs and interact with molecules in the bulk of the liquid. The reactions which ·OH, H·, e^-_{aq} may undergo, whether in the spurs or in the bulk medium, are shown in Table III. The relative number of radicals which combine to give molecular products or which do not combine and form radical products is dependent on the LET of the radiation. For water with pH = 7 irradiated with γ-rays, the G value (number of molecules of product formed per 100 eV of radiation energy absorbed) for various species is shown in Table IV (Spinks and Woods, 1976).

2.1.4 Direct and Indirect Action of Radiation

When an aqueous solution is exposed to ionizing radiation, energy may be absorbed by both solute and solvent molecules. Excited molecules

TABLE III

Reactions of e^-aq, $\cdot OH$, and $H\cdot$

I. Reactions of e^-aq

A. Reactions with oxidizing agents (diffusion controlled)

$e^-aq + H_2O_2 \rightarrow \cdot OH + OH^-$

$e^-aq + O_2 \rightarrow O_2^-$

$e^-aq + \cdot OH \rightarrow OH^-$

B. Nucleophilic substitution

$e^-aq + H_2O \rightarrow H\cdot + OH^-$

$e^-aq + RX \rightarrow R\cdot + X^-$

II. Reactions of $\cdot OH$

A. Hydrogen abstraction

$\cdot OH + H_2 \rightarrow H_2O + H\cdot$

$\cdot OH + RH \rightarrow H_2O + R\cdot$

$\cdot OH + CH_3OH \rightarrow H_2O + \cdot CH_2OH$

B. Addition

$\cdot OH + RH \rightarrow H\dot{R}OH$

$\cdot OH + R_2C = CR_2 \rightarrow R_2OHC-\dot{C}R_2$

C. Oxidative electron transfer

$\cdot OH + M^{z+} \rightarrow M^{(z+1)+} + OH^-$

$\cdot OH + X^- \rightarrow X\cdot + OH^-$

D. Displacement

$\cdot OH + RI \rightarrow ROH + I\cdot$

E. Radical combination

$\cdot OH + \cdot OH \rightarrow H_2O_2$

$\cdot OH + H\cdot \rightarrow H_2O$

Table III (Continued)

III. Reactions of H·

A. Hydrogen abstraction

$H\cdot + RH \rightarrow R\cdot + H_2$

$H\cdot + CH_3OH \rightarrow \dot{C}H_2OH + H_2$

$H\cdot + CH_3CH_2OH \rightarrow CH_3\dot{C}HOH + H_2$

B. Addition

$H\cdot + O_2 \rightarrow HO_2\cdot$

$H\cdot + R_2C{=}CR_2 \rightarrow R_2HC{-}\dot{C}R_2$

C. Reductive electron transfer

$H\cdot + M^{(z+1)+} \rightarrow M^{z+} + H^+$

D. Reactions with oxidizing agents

$H\cdot + H_2O_2 \rightarrow OH\cdot + H_2O$

E. Radical combination

$H\cdot + \cdot OH \rightarrow H_2O$

$H\cdot + H\cdot \rightarrow H_2$

TABLE IV

Product yield (G values) of various radicals and molecules in water at pH = 7 for low LET radiation (x-rays or γ-rays) (Spinks and Woods, 1976).

H_2	0.45	molecular products
H_2O_2	0.68	
e^-_{aq}	2.63	radical products
$\cdot OH$	2.72	
$H\cdot$	0.55	
HO_2	0.026	

and ions will thus be produced from both solute and solvent molecules. If the energy of the radiation is deposited directly in the solute, this is referred to as the direct effect. This is in contrast to the indirect effect, in which energy is absorbed by solvent molecules. Excited species are formed which subsequently react with solute molecules. If the solution is dilute, the indirect effect predominates. The reactions which occur will be those resulting from the interactions of reactive species from the solvent with each other or with any solute that is present. The extent of indirect action is dependent on the yield of reactive species of the solvent, which is water for most biological systems. Thus the number of solute molecules inactivated is independent of the concentration in the absence of chain reactions. The percentage of inactivation, however, decreases with increasing concentration. This is referred to as the dilution effect. If the action of the radiation is direct, the number of molecules which have been inactivated will depend on the number of molecules present, and is therefore proportional to the concentration. The percentage inactivation will thus be constant for a given dose.

2.1.5 Radiation Protection and Sensitization

Although radiation damage produced in biological systems originates from events which occur at the molecular level such as excitation, ionization, and radical formation, it is possible to modify that damage by altering the chemical or physical environment of the system. The ultimate biological damage may be either enhanced or reduced by the addition of radiation sensitizers or protectors respectively.

Radiation protectors at the molecular level are divided into two categories, competitive and restitutive.

Competitive protection refers to the ability of various chemical compounds to compete with biomolecules for free radicals. The amount of protection depends on the relative number of the competing molecules and on their reaction constants with the available free radicals. Protectors of this type are referred to as radical scavengers. Radical scavengers reduce the effect of indirect action by reacting quickly with free radicals and thus inactivating them. Alcohols, amines, and sulphydryl compounds are good ·OH radical scavengers. Sulfhydryl compounds will also scavenge H· radicals.

$$H\cdot + RSH \rightarrow RS\cdot + H_2$$

$$\cdot OH + RSH \rightarrow RS\cdot + H_2O$$

$$2RS\cdot \rightarrow RS\text{-}SR$$

Restitutive protection does not alter the number of damaged molecules or primary lesions in biomolecules. Instead, some of the sublethal damage is restituted or repaired by a protective agent, giving a net protective effect. By the hydrogen donation mechanism (Alexander and Charlesby, 1955), a damaged biomolecule, M·, is restituted by the transfer of a hydrogen atom from a protector, commonly a sulfhydryl compound, to the molecular radical.

$$M\cdot + RSH \rightarrow MH + RS\cdot$$

The sulfhydryl radical will be less damaging to the biological system than the molecular radical, and may recombine with a hydrogen radical.

Oxygen molecules, which are electronegative, may also scavenge electrons and hydrogen radicals.

$$O_2 + e^-_{aq} \rightarrow O_2^-$$

$$O_2 + H\cdot \rightarrow HO_2\cdot$$

In aqueous solutions, the oxygen may protect if e^-_{aq} and H· are important in an inactivation process. In general, however, the radiation sensitivity of biological systems and biomolecules, specifically enzymes and nucleic acids, irradiated in air or oxygen, is usually higher than when irradiated under anoxic conditions, such as in an inert gas or vacuum.

A radiation sensitizer is defined as an agent which increases a given radiation response when present during the irradiation of the material. Radiation sensitization may refer to the inhibition of a naturally occurring protective effect, or to a true sensitization which results from an intermolecular energy transfer or an increase in the number of free radicals present. The latter is a general enhancement of the indirect effect. Radicals produced from indirect action may react with sensitizers in competition with repair mechanisms. The oxygen fixation mechanism (Alexander and Charlesby, 1954) is an example of this. Molecular radicals (M·) may react with molecular oxygen, which is present during the irradiation, to form a peroxy radical. This radical is irreparable and is a precursor of radiation damage. This process competes with hydrogen transfer from sulfhydryl compounds to the M· radical.

$$M\cdot + O_2 \rightarrow M\text{-}O\text{-}O^{\cdot}$$

$$M\cdot + RSH \rightarrow RS\cdot + MH \text{ (competition)}$$

Therefore, if oxygen competes with restitution processes in a macromolecule, enhancement of sensitivity to radiation occurs. This is not a true sensitization since there is no increase in the number of free radicals, but is due to the reduction or cessation of restitution

processes which proceed in the absence of oxygen. The relative insensitivity of hypoxic cells to radiation killing is well known in tumor radiotherapy (Gray et al., 1953).

Within the past several years, radiofrequency and microwave radiation with various frequencies has been shown to sensitize mammalian cells in vivo and in vitro to ionizing radiation by heating the tissue. This has been done clinically with cancer patients with various types of tumors. The hypoxic malignant cells have more thermal sensitivity than normal cells (Cavaliere et al., 1967; Mondovi et al., 1969) and thus cannot repair sublethal damage incurred by radiation when the tissue temperature is elevated.

In one instance, it has been claimed that microwave radiation has had a protective effect on mammalian cells in vitro. Exposure to 100 mW/cm^2, 2800 MHz pulsed microwaves decreased the mortality from subsequent x-ray exposure (Baranski and Czerski, 1976). This, however, has not been substantiated.

2.2 Effect of Ionizing Radiation on Biological Macromolecules

2.2.1 Biological Macromolecules

Cells contain hundreds of different categories of organic cellular components, three of which are common to all types of cells. These are carbohydrates, proteins, nucleic acids, and all their derivatives. In cells, these molecules are often very large and are referred to as macromolecules. The most common macromolecules in cells are polysaccharides, proteins, conjugated proteins such as lipoproteins, glycoproteins, or nucleoproteins, and nucleic acids such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

The biological effect of ionizing radiation may be studied at various levels. At the molecular level, the chemical and physical processes which have occurred as a result of irradiation may cause a variety of structural changes in macromolecules which may lead to an alteration in function. The macromolecule may be degraded or broken into smaller units. In molecules with repeating units, the breaks usually occur at the same bond, which result in many smaller molecules with a similar chemical composition to the original molecule. This phenomenon has suggested the occurrence of intramolecular energy transfer; that is, energy absorbed at any spot in a molecule may be transmitted down the molecule to the weakest bond.

Another common structural change is cross-linking, which may be intramolecular or intermolecular. Intermolecular cross-linking occurs when two or more molecules are joined together. As the process of cross-linking continues, an extensive network of molecules or a gel is formed which can no longer function normally and may not even still be soluble in the medium. Intramolecular cross-linking occurs by radical-radical recombination or by radical addition to unsaturated structures within the same molecule. This process can occur within long flexible molecules which continuously alter their conformation. Intramolecular cross-linking pulls the molecule together so it occupies a smaller volume in solution, thus reducing the viscosity of the solution without any change in molecular weight.

Another structural change is the disruption of the secondary or tertiary structure of macromolecules. Since many macromolecules maintain rigid conformation by hydrogen bonding, disruption of the weak hydrogen bonds by radiation can alter macromolecular structure and hence function.

2.2.2 Proteins

Proteins are important in almost all biological processes. They function in such processes as enzyme catalysis, immune protection, transport and storage of ions and molecules, co-ordinated motion, and control of growth, differentiation, and metabolism. The basic structural units of proteins are amino acids which vary in size, shape, charge, chemical reactivity, and hydrogen bonding capacity. Amino acids exist in solutions in two forms, ionized or unionized (Figure 2.6). At neutral pH they exist as zwitterions; the carboxyl group is dissociated ($-COO^-$) and the amino group is protonated ($-NH_3^+$) giving a net electrical charge of zero. In acid solutions, the carboxyl group is un-ionized (-COOH) and the amino group is ionized ($-NH_3^+$) whereas in alkaline solutions the reverse occurs ($H_2N-CHR-COO^-$).

Amino acids are joined together by peptide bonds to form peptides or polypeptides which are chains with over 100 amino acid residues. A protein usually contains one or more polypeptide chains and ranges in molecular weight from 10^3 to 10^6 daltons. The function of the protein is specified by its conformation or the three-dimensional arrangement of atoms in the molecule. The primary structure refers to the nature and sequence of amino acid residues in the chain, whereas secondary and tertiary structure refer to the polypeptide chain configuration and the subsequent folding of the polypeptide chain respectively. This coiling and twisting of a polypeptide results in a more compact molecule which is held together by disulfide bonds, ionic bonds, van der Waals forces and hydrogen bonds. The resultant conformation is specific for each protein and specifies its function.

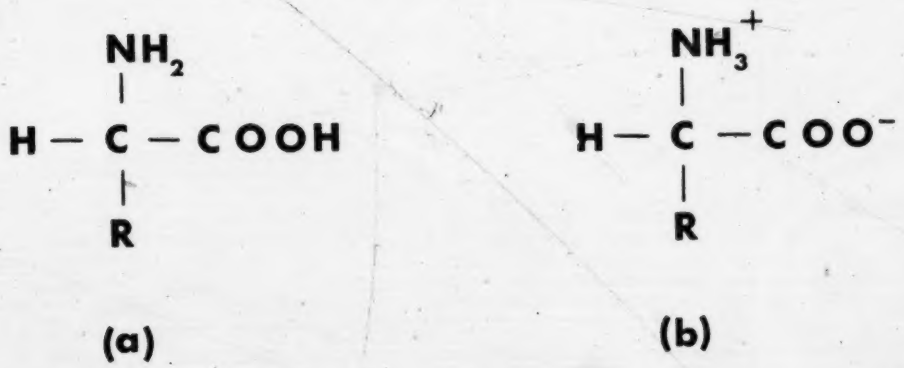


Fig. 2.6 Structure of an amino acid. R is the side chain.

(a) un-ionized form of an amino acid

(b) dipolar ion or zwitterion

The most radiosensitive portion of an amino acid is the amino group. In a protein, however, the amino group is bonded to a carboxyl group and so is not easily removed from the molecule. Therefore, the side chain of the amino acid is the most radiosensitive portion of a protein molecule. Depending on the chemical composition of the side chain, various reactions may occur, such as a break in a covalent or hydrogen bond and radical addition (e.g., hydrogen atom or hydroxyl radical) to aromatic structures in tyrosine or tryptophan residues.

After the irradiation of amino acids with aliphatic hydrocarbon side chains (glycine, valine, leucine, isoleucine, proline, and alanine) in an oxygenated aqueous solution, the products found were hydrogen peroxide, α-keto acids and aldehydes (carbonyl groups), and ammonia. The ammonia yield was less for amino acids with sulfur side chains (methionine and cysteine) or amide side chains (asparagine and glutamine) (Garrison, 1964). The most radiosensitive amino acids are those with sulfur side chains or aromatic side chains (tyrosine, tryptophan, and phenylalanine). Amino acids with aromatic side chains are highly reactive to OH· radicals and moderately reactive to ·H radicals and solvated electrons. Cysteine is highly reactive to all reactive species, H· and ·OH radicals, and solvated electrons. Cysteine radicals which are formed after irradiation may dimerize and form cystine units.

Irradiation of a simple peptide chain in an oxygenated, aqueous solution, yields amino acid degradation products, and amide and carbonyl groups. This indicates that peptide bonds have been broken (Garrison, 1964).

After irradiation, proteins may lose their ability to function due to breakage of peptide bonds, chemical change in a side chain, or from

breakage of a disulfide or hydrogen bond responsible for maintaining the secondary and tertiary structure of the molecule. When an ionizing particle passes through biological material, the sudden appearance of an electrical charge will disrupt the local electron distribution. This leads to breakage of weak hydrogen bonds. If enough hydrogen bonds are broken, this is sufficient to cause an alteration in the conformation of a protein and may lead to a drastic change in chemical reactivity (Casarett, 1968).

The reactivity per mole of protein molecules, in general, is much higher than most of the highly reactive amino acids. This is due to the presence of many reactive acid residues in a protein molecule. However, this is not true for all proteins. Globular proteins such as lysozyme and ribonuclease are less reactive than the sum of the reactivities of their amino acid residues. This difference is most likely due to the folding of the polypeptide chains and in the distribution of charge on the protein (Collinson et al., 1950). This has been substantiated by the fact that at an elevated temperature ribonuclease unfolds and there is a fourfold increase in its reactivity to hydrated electrons. This is most likely due to the exposure of hidden disulfide bridges which are very easily broken by hydrated electrons.

The radiation sensitivity of a protein is complicated by its secondary and tertiary structure. Amino acid residues of the same type in one protein molecule may have different radiation sensitivities depending on their location and environment in the molecule. Residues on the surface are more accessible to reactive species than those within the protein, whereas amino acids associated with an active site are potentially very reactive. The amino acid residues and ionic environment

surrounding an amino acid will also affect its radiation sensitivity. Hydrogen bonds between amino acids, which determine the tertiary structure of a protein, may affect the radiosensitivity around a peptide bond (Garrison and Weeks, 1962).

Enzymes, which are proteins, catalyze reactions between two molecules. They are highly specific for a given reaction and substrates. The active site of an enzyme is the region which binds the substrates and adds residues that help make or break bonds. The radiolysis of enzymes may render the active site inactive, which would cause a loss of function for the entire molecule. Reactive species may alter an amino acid residue on the enzyme surface and form a carbon (C·) or sulfur radical (S·) by hydrogen abstraction (Luse, 1964). Intramolecular energy transfer may lead to rupture of weak covalent or ionic bonds. This would cause an alteration in conformation (Luse, 1964). Both mechanisms would cause inactivation of the active site, one by radical formation, the other by disruption of conformation.

Enzymes irradiated dry (i.e., ribonuclease or lysozyme), with doses in the kilorad range, and then dissolved in water, show an increase in relative viscosity and denatured products, and a decrease in the tightly bound amide hydrogen atoms (Okada, 1970). This suggests that a change in conformation has taken place which will affect the active site, rendering it inactive. Possible mechanisms are by intermolecular cross-linking, breakage of disulfide bonds, polypeptide chain breaks, and conformational change of side chains of enzyme molecules (Okada, 1970).

Enzymes or proteins which contain an inorganic prosthetic group may be inactivated by damage to the prosthetic group. A heme group,

consisting of an organic part and an iron atom, is found in enzymes and proteins such as hemoglobin. This group may be inactivated by radical attack, causing loss of function for the entire molecule.

To cause inactivation of an enzyme in vivo or in vitro, doses must be in the kilorad range. This contrasts with the doses needed to kill mammalian cell populations in vivo or in vitro, of a few hundred rads. In vivo, it is difficult to tell if alteration of enzymatic activity has been caused by radiation damage to the enzyme or by damage to other cell sites, such as membranes. Radiation damage to enzymes may be secondary to the other radiation damage. This is consistent with the fact that larger doses are needed in vitro to cause a detectable change in enzymatic activity than are needed in vivo.

2.2.3 Nucleic acids

Nucleic acids are long unbranched macromolecules consisting of nucleotides joined by 3' - 5' phosphodiester bonds. A nucleotide consists of a sugar, phosphate, and base. There are two important nucleic acids found in biological systems, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

DNA is the structural unit of heredity in prokaryotic and eukaryotic cells. All cellular DNA consists of two long helical polynucleotide chains coiled together as a double helix. The backbone of each strand consists of deoxyribose sugar units connected by phosphodiester bridges, which are found on the outside of the double helix. The purine and pyrimidine bases are joined to the sugar units, and are found on the inside of the molecule. The two strands are linked together by hydrogen bonding between the base pairs. Guanine is always paired with

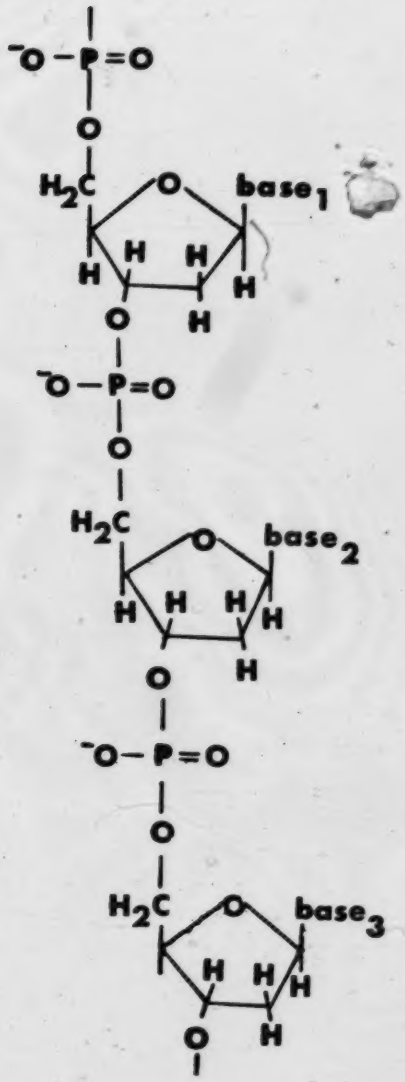
cytosine, and thymine with adenine (Figure 2.7). Thus one strand is the complement of the other. The precise sequence of bases in the polynucleotide is the code for genetic information, where three bases code for one amino acid.

RNA is similar to DNA in that it is a long chain of nucleotides. However, RNA molecules are almost always single-stranded except for regions of double-helical structure formed by hairpin loops. The sugar unit in RNA is ribose instead of deoxyribose, and the pyrimidine base uracil replaces thymine and pairs with adenine.

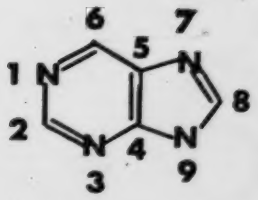
Nucleic acids are points of extreme radiosensitivity due to the specialized function of DNA in cells. Nucleic acids have a high specificity of structure so that small alterations in the molecule may lead to alterations in function. Since they code for genetic information in their sequence of bases, radiation-induced mutations may be passed on to subsequent generations (Latarjet, 1972) and thus any radiation damage may be magnified.

Nucleic acids control many important cellular processes. Radiation damage may lead to a decrease in any of these processes in the irradiated cell. This indicates that nucleic acids are likely target molecules for radiation effects on these cellular processes (Okada, 1970). This is substantiated by the fact that the radiation-induced inhibition of a function decreases proportionally to the repair of a radiation-induced lesion in a nucleic acid (Latarjet, 1972).

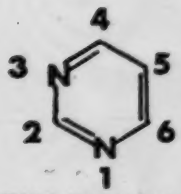
The radiolysis of DNA in aqueous systems may lead to many different types of radiation-induced damage in DNA molecules. These are summarized as follows.



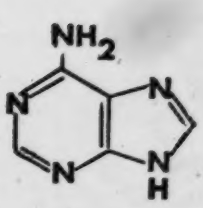
Structure of part of a DNA chain



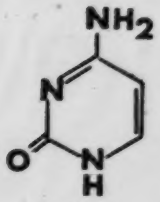
PURINE



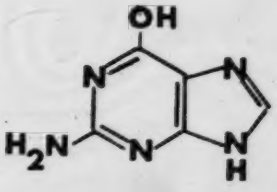
PYRIMIDINE



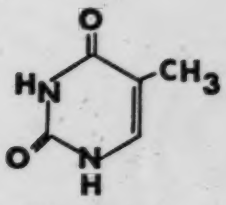
ADENINE



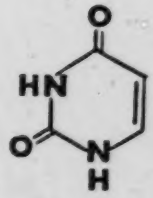
CYTOSINE



GUANINE



THYMINE



URACIL

The purine and pyrimidine bases which comprise DNA and RNA.

Fig. 2.7 DNA structure.

1. Loss or change of a base
2. Hydrogen bond breakage between base pairs
3. Changes in the sugar
4. Single or double strand breaks
5. Cross-linking within the helix or within DNA-protein complex
6. Intermolecular cross-linking between DNA molecules

During the physiochemical stage of the radiolysis of DNA, free radicals are formed which can be observed using ESR-spectroscopy. Water radicals are formed which attack the DNA molecule forming base and sugar radicals. Base radicals are formed to a greater extent than sugar radicals.

Pyrimidine and purine radicals will be formed by hydrogen addition predominantly at the 5,6 double bond in pyrimidines, and at the 2 or 8 carbon in purines (Dertinger and Jung, 1970). Deoxyribose sugars form a radical by loss of a hydrogen atom and subsequent ring-opening. The hydrogen atoms that have split off the sugar may attack the nucleoside or nucleotide, increasing the yield of radicals by an indirect effect (Dertinger and Jung, 1970).

$$MH + H\cdot \rightarrow MH_2\cdot$$

Decomposition of pyrimidines (G = 1.9-2.1) occurs to twice the extent as that of purines (G = 1.1-1.3) which are irradiated in aqueous solutions (Scholes, 1968). Pyrimidine bases undergo OH· radical attack mostly at the 5,6 double bond. Under aerobic conditions, a pyrimidine hydroxy-hydroperoxide is formed. Cytosine and uracil hydroxy-hydroperoxides are very unstable and decompose rapidly (Schwiebert and Daniels, 1971). Purines irradiated under anerobic conditions undergo

OH· and ·H addition to the 7,8 double bond, rupturing the imidazole ring. Under aerobic conditions, peroxidation of the 5,6 double bond occurs. The resulting hydroxy-hydroperoxide is unstable. In the presence of oxygen, twice as many bases will be destroyed as under anaerobic conditions, and thymine is destroyed more rapidly than the other bases due to the inherent reactivity of thymine.

Free bases are more radiosensitive than bases which are bound in double-stranded polynucleotides. This is due to the fact that they are in the interior of the molecule and there is a decrease in probability of radical attack. Under comparable experimental conditions, the destruction of free bases by ionizing radiation is four times as likely to occur as loss of a base from irradiated DNA (Hems, 1960). Since the base sequence in DNA carries the genetic information, any alteration in a base which leads to a difference in the base sequence may result in alterations in an entire cell or organism.

Hydrogen bonds between base pairs in DNA may be broken by ionizing radiation. These may be reformed immediately, or, if sufficient number of bonds have been broken at the same time, the two polynucleotide chains may become separated. Radicals may then attack the bases and irreparable damage may occur.

If the four nucleotides are irradiated in an aqueous solution, approximately 20% of the water radicals will react with the sugar group and 80% with the bases (Scholes et al., 1960). Radical attack on the deoxyribose sugar in DNA may lead to hydrolysis of the N-glycosidic bond, releasing undamaged bases, or to a break in the nucleotide chain, which may release 3'- or 5'- monophosphates. These single strand breaks in a 0.1% solution of double-stranded DNA have a G-value of 0.4, whereas the

G-value for base destruction is 1.7 (Collyns et al., 1965). In cells, most single strand breaks will rejoin but some single strand breaks may become peroxidized at one end if oxygen is present and therefore are unable to rejoin.

Double chain breaks will occur if there is a break in both strands less than five nucleotides apart. This may occur when two single strand breaks occur in the same vicinity or when a particle with a high LET produces a double strand bread, which requires about 600 eV (Setlow and Setlow, 1972). Double strand breaks may also be caused by a spur produced by low LET radiation (Cole et al., 1974).

Cross-linking between and within DNA molecules may occur when reactive sites are formed at a strand break. Two reactive sites may then combine. This has been shown to occur in the production of thymine dimers by ultraviolet radiation, but this may occur as well with ionizing radiation, with formation of cystine dimers.

$$R\cdot + R\cdot \rightarrow R - R$$

DNA which is complexed with proteins is less sensitive to chain breakage or base destruction. The protein competes with the DNA for water radicals and may possibly shield the DNA from the reactive species. DNA may form cross-links with the protein if reactive sites are formed on both molecules.

The biochemical alterations which may occur in DNA as a result of exposure to ionizing radiation will be ultimately manifested as biological responses. A base change in DNA will lead to mutations which

are passed on to subsequent generations. Single and double strand breaks and cross-linking may lead to chromosomal aberrations which may also lead to deleterious effects in later generations.

CHAPTER III

NON-IONIZING RADIATION

3.1 Microwave Biophysics

3.1.1 Interaction of Microwaves with a Biological System

(A) Physical characteristics of biological systems

The attenuation of microwave radiation by a biological system is a complex process. However, the analysis of plane wave attenuation using the macroscopic physical properties of a biological material has produced several useful concepts. These will be outlined in detail in later sections.

Biological materials can be characterized by three physical parameters: the conductivity (σ), the electrical permittivity (ε), and the magnetic permeability (μ). These parameters refer to the macroscopic properties of the biological system. Characterizing a material with these parameters implies a dissipative mode of interaction with an electromagnetic field. Two processes may occur in which energy is removed from the field: motion of free charges or ions (conduction loss) and molecular rotation (dielectric loss). Biological systems which have a finite conductivity are referred to as lossy dielectrics.

When dealing with a biological system, the assumption is made that all charges in the system have reached their equilibrium positions and are fixed in space. Inside the system, the net electric field ($\bar{E}$) is zero, and from Gauss' law, the net charge density (ρ) must also equal zero.

The conductivity (σ) of a material is a measure of the electronic reactivity of that material or a measure of the free charge density. This indicates to what extent a material conducts electricity. The conductivity is found by the relationship:

$$\bar{J}_f = \sigma \bar{E} \tag{1}$$

where $\bar{J}_f$ = free charge current density (amps/m^2)

$\bar{E}$ = electric field intensity (volts/meter)

The conductivity is measured in 1/(ohms·meter) which is defined as mhos/meter.

The dielectric constant or relative permittivity (K_e) indicates to what extent the electric lines of force pass through a substance, more readily or less readily, than they would pass through an equivalent volume of a vacuum. The dielectric constant is a dimensionless parameter and is the ratio of the flux density of electric lines of force through a material to that of a vacuum. The dielectric constant is defined by:

$$K_e = \frac{\varepsilon}{\varepsilon_o} \tag{2}$$

This permittivity of free space (ε_o) is a constant with the value 8.85×10^{-12} farads/meter. In general, insulators have a dielectric constant greater than one, thus more electric lines of force pass through an insulator than through an equivalent volume of free space.

Both the conductivity and the permittivity are frequency and temperature dependent. In the microwave range, $\Delta\sigma/\sigma$ changes by +2% with each degree centigrade and $\Delta\varepsilon/\varepsilon$ changes by -0.5% with each degree

centigrade (Johnson and Guy, 1972). The frequency dependence is more complex and will be discussed in section 3.1.2 (B).

The permeability (μ) indicates the ease in which a magnetic field may be set up in a material. If a material substance was placed in a magnetic field with a given flux density, the flux density through the material would change. The relative permeability (K_m), a dimensionless parameter, is the ratio of the magnetic flux density through the material to that of a vacuum. The permeability of free space (μ_o) is a universal constant with the value of 1.257×10^{-6} henry/meter.

Biological systems are generally considered to be diamagnetic materials since most of the atoms and molecules in a biological object do not possess a permanent magnetic moment. This difference between the permeability of biological tissue and that of free space is less than 0.01% in most cases, so it is assumed within experimental error that $\mu = \mu_o$ and $K_m = 1$ (Cleary, 1970).

(B) The wave equation in biological media

The general solution to the wave equation (derived from Maxwell's equations) for a plane wave propagating in an uncharged medium ($\rho = 0$) with a finite conductivity ($\sigma > 0$) is given by (Neuder, 1978):

$$\bar{E} = \hat{i}\, E_o\, e^{i(Kz-\omega t)} \qquad (3)$$

$$\bar{H} = \hat{j}\, H_o\, e^{i(Kz-\omega t)} \qquad (4)$$

where $\bar{E}$ and $\bar{H}$ represent the electric and magnetic fields respectively inside the biological system.

The square of the propagation constant (K) is defined as (Neuder, 1978):

$$K^2 = \mu\varepsilon\omega^2 + i\omega\mu\sigma \tag{5}$$

where σ, ε, and μ are the macroscopic electric properties of the material and ω is the angular frequency ($\omega = 2\pi\nu$).

Since K is a complex quantity, it may be written as:

$$K = \alpha + i\beta \tag{6}$$

The real and imaginary parts of the propagation constant are obtained by combining equations (5) and (6) (Shadowitz, 1975).

$$\alpha = \omega\sqrt{\frac{\mu\varepsilon}{2}}\left[\sqrt{1+\left(\frac{\sigma}{\varepsilon\omega}\right)^2}+1\right]^{1/2} \tag{7}$$

$$\beta = \omega\sqrt{\frac{\mu\varepsilon}{2}}\left[\sqrt{1+\left(\frac{\sigma}{\varepsilon\omega}\right)^2}-1\right]^{1/2} \tag{8}$$

Using equations (3) and (6), the electric field may be written as:

$$\bar{E} = \hat{i}\, E_o\, e^{-\beta z}\, e^{i(\alpha z-\omega t)} \tag{9}$$

The term $e^{-\beta z}$ indicates exponential damping of the wave due to the finite conductivity of the medium.

The distance at which the wave amplitude will decrease by a factor of 1/e can be used to define a characteristic depth of penetration (d) where $d = 1/\beta$. This is referred to as the skin depth of the material and is measured in centimeters. At this distance, the power density will have decreased to 37% of its initial value at the surface of the object,

and 87% of the total energy of the electromagnetic wave will have been dissipated in the tissue.

The magnetic field ($\bar{H}$) can also be written as a function of α and β (Panovsky and Phillips, 1955):

$$\bar{H} = \frac{\hat{k} \times \bar{E}}{K\mu\omega} (\alpha + i\beta) \tag{10}$$

This equation demonstrates that there will be a phase difference between the electric and magnetic fields depending on the physical properties of the medium which determine α and β.

At the interface between free space and a biological medium, the frequency of the electromagnetic radiation does not change as the wave penetrates the material. Since the velocity of propagation of an electromagnetic wave in free space is $c = \nu\lambda$ ($c = 3 \times 10^8$ m/sec), the relationship between the free space wavelength (λ_o) and the wavelength in the medium (λ) is:

$$\frac{\lambda_o}{\lambda} = \frac{c}{v} \tag{11}$$

Since the wave vector is described by $\alpha = \omega/v$, the ratio λ/λ_o can be rewritten as:

$$\frac{\lambda}{\lambda_o} = \frac{\omega}{c\alpha} \tag{12}$$

Thus, the wavelength in the medium can be described by the relationship:

$$\lambda = \frac{2\pi}{\alpha} \tag{13}$$

The wavelength in the medium is a function of the electric properties of the medium and will differ in various dielectric media.

The ratio of the imaginary to real parts of the square of the propagation constant (K^2) from equation (5) is referred to as the loss tangent, tan δ. This is given by:

$$\tan \delta = \frac{\sigma}{\omega \varepsilon} \tag{14}$$

This equation gives the ratio of conduction currents to displacement currents. The loss tangent is a direct indicator of the degree of energy absorption in the medium. The loss tangent in biological systems will be discussed in section 3.1.2 (B).

For the case of a plane wave, the electric and magnetic field vectors are perpendicular to each other and to the direction of propagation of the wave. The scalar components of the electric and magnetic field vectors are related to the intrinsic impedance of the medium (Z), which is the ratio of voltage to current travelling together in a particular direction when there is no reflected wave travelling in the opposite direction. This relationship is given by:

$$Z = \frac{|\bar{E}|}{|\bar{H}|} = \left(\frac{\mu}{\varepsilon} - \frac{i\sigma}{\omega}\right)^{-1/2} \tag{15}$$

In a non-conducting dielectric, Z is equal to $\sqrt{\mu_o/\varepsilon_o}$. In air, Z = 377 ohms and the relationship between $\bar{E}$ and $\bar{H}$ is $\bar{E} = 377\ \bar{H}$.

For a time-varying electromagnetic wave propagating in free space, the electric and magnetic fields are oriented perpendicular to each other such that at any point in time and space, their vector cross product ($\bar{E} \times \bar{H}$) results in the Poynting vector ($\bar{S}$). This may also be expressed in terms of the magnetic induction ($\bar{B}$) as $S = (\bar{E} \times \bar{B})/\mu_o$, where μ_o is the permeability of free space (Figure 3.1). The magnitude

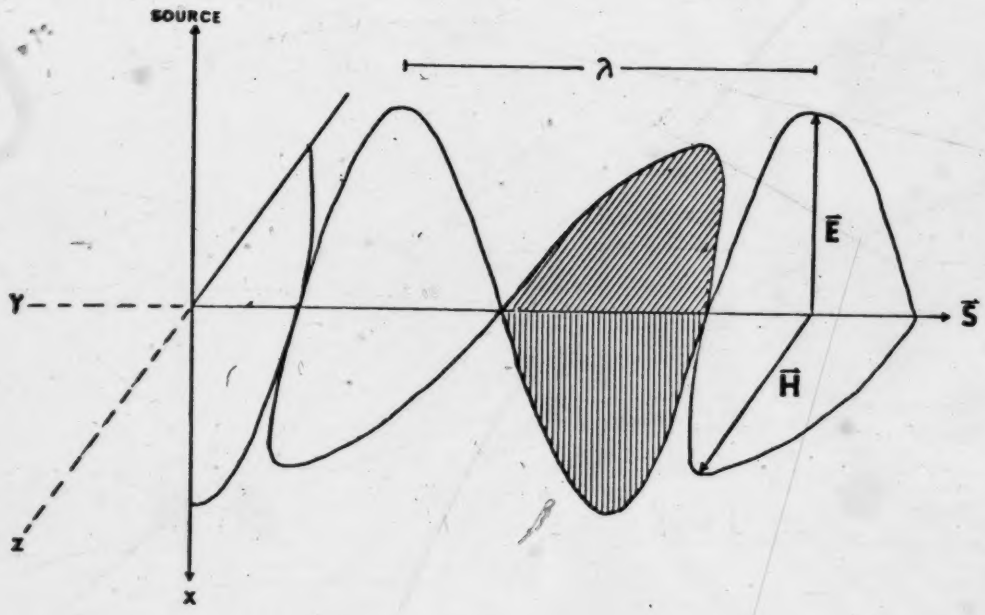


Fig. 3.1 Relationship between the electric field component $\vec{E}$ and the magnetic field component $\vec{H}$ with the resultant vector cross product, the Poynting vector $\vec{S}$. The direction of propagation is along the y axis.

of the Poynting vector is equal to the energy crossing a unit area per unit time and its direction is parallel to the direction of the wave vector. Generally the time-averaged value of the Poynting vector is used. This is given by (Shadowitz, 1975):

$$\langle \bar{S} \rangle = \mathrm{Re}\left(\frac{1}{2\mu_o} \bar{E} \times \bar{B}^* \right) \qquad (16)$$

where B* is the complex conjugate of B.

Electromagnetic wave energy is conventionally measured in terms of power density, which corresponds to energy flux per unit area. This exposure for microwaves is generally expressed in milliwatts per square centimeter (mW/cm^2) or microwatts per square centimeter ($\mu W/cm^2$).

The absorbed power density in the medium as the result of the electromagnetic wave penetration is related to the electric field strength (E_o) and the conductivity (σ) by the equation (Neuder, 1978):

$$P = \frac{\sigma}{2} \left| E_o \right|^2 \quad (mW/cm^3 \text{ or } W/m^3) \qquad (17)$$

Since the electric field strength is a function of the electrical properties of the tissue and the boundary conditions, the absorbed microwave energy is also dependent on the electrical and physical properties of the tissue.

3.1.2 Microwave Absorption by a Biological System

(A) General absorption characteristics

In the microwave region where $\varepsilon\omega << \sigma$, equations (7) and (8) reduce to:

$$\alpha \simeq \omega \sqrt{\frac{\varepsilon\mu}{2}} \sqrt{\frac{\sigma}{\varepsilon\omega}} = \sqrt{\frac{1\mu\sigma\omega}{2}} \tag{18}$$

$$\beta \simeq \sqrt{\frac{1\mu\sigma\omega}{2}} \tag{19}$$

When $\varepsilon\omega << \sigma$, the skin depth (d) is given by the equation:

$$d = \frac{1}{\beta} = \sqrt{\frac{2}{\mu\sigma\omega}} \tag{20}$$

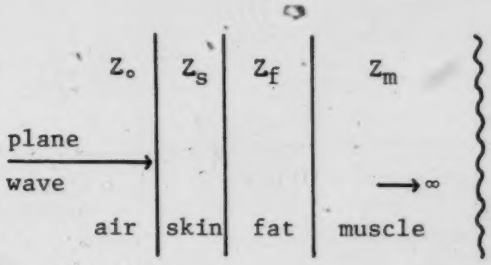
The skin depth, or penetration depth, decreases with increasing frequency. Thus, the energy absorption by the medium increases with the frequency of the electromagnetic wave.

Since α and β are approximately equal for microwave absorption by biological materials, the phase angle as described by equation (10) will be 45°. In this case, the magnetic field lags the electric field.

The depth distribution of the absorbed microwave energy varies greatly depending on the frequency of the radiation and on the dielectric and physical properties of the tissue. Microwave radiation with a frequency less than 3000 MHz penetrates more deeply into the body and thus the energy absorption is more diffuse. The absorption of microwave energy with intermediate frequencies (900-3000 MHz) depends on many factors such as the thickness, spatial orientation, and composition of the material, and the texture of the surface of the material.

Previously, the penetration of microwaves into a planar, semi-infinite layer of biological media has been discussed. In order to determine reflection coefficients at tissue interfaces, a model has been developed which consists of three layers of tissue: skin, fat, and muscle.

This semi-infinite planar surface is irradiated by a plane wave. The skin and fat layers are finite, but the muscle layer, in which all microwave energy will be dissipated, is thus considered infinite (Baranski and Czerski, 1976).



One limitation of the three layer model is that the radius of curvature of the target surface must be sufficiently larger than the wavelength of the radiation so that the object is approximately planar. This precludes the use of this model in determining the energy absorption in rats, mice, or other smaller animals with microwave radiation of centimeter or decimeter wavelength. Also, this model implies that all successive body layers after the muscle layer have the same dielectric properties as muscle, and form planar layers. This must be taken into account when calculating the actual energy absorption by the biological object.

At tissue interfaces, discontinuities in the dielectric properties occur resulting in field perturbations such as production of standing waves. The ratio of the amplitudes of the reflected wave (E_r) to the incident wave (E_i) will give an indication of the field penetration into a tissue. For a plane electromagnetic wave normal to a semi-infinite planar surface, this is given by the reflection coefficient (ρ).

The reflection coefficient for wave transmission between any two tissue layers with different complex permittivities, ε_1 and ε_2, and thickness greater than skin depth is given by:

$$\rho = re^{i\phi} = \frac{\sqrt{\varepsilon}_1 - \sqrt{\varepsilon}_2}{\sqrt{\varepsilon}_1 + \sqrt{\varepsilon}_2} \tag{21}$$

Reflection coefficients at interfaces between tissues with high and low water contents are given in Tables V and VI respectively.

From Tables V and VI, it can be seen that the power absorbed in tissues with high water content is many times greater than in tissues with low water content. The amount of power absorbed by a tissue is characterized by the depth of penetration of a given frequency of microwave radiation. In tissue with low water content, such as bone or fat, low frequency electromagnetic fields do not interact significantly with the tissue, and thus the depth of penetration is large. The reverse is true for high frequency electromagnetic waves in a tissue with high water content, such as skin or muscle. In this case, the depth of penetration is small, and the incident electromagnetic energy is dissipated quickly.

A wave propagating in a tissue with low water content, incident on a tissue with high water content, is reflected nearly 180° out of phase with the incident wave. This results in production of a standing wave with its intensity minimum near the interface. When a wave propagating in a tissue with high water content is incident on a tissue with low water content, the reflected wave is in phase with the incident wave and an intensity maximum occurs near the interface. In both cases, the wave must be incident on tissue with thickness greater than the

TABLE V

Characteristics of electromagnetic wave propagation in tissues of high water content (such as muscle or skin) (Johnson and Guy, 1972)

Frequency ν (MHz)	Wavelength λ_o/λ (x 10^{-2} m)	Depth of penetration $1/\beta$ (x 10^{-2} m)	Conductivity σ (mho/m)	Dielectric constant K_e	Reflection coefficient at interface			
					Air/muscle		Muscle/fat	
					r	ϕ	r	ϕ
100	300/27	6.66	0.889	71.7	0.881	+175	0.650	-7.96
200	150/16.6	4.79	1.28	56.5	0.844	+175	0.612	-8.06
300	100/11.0	3.89	1.37	54	0.825	+175	0.592	-8.14
433	69.3/8.76	3.57	1.43	53	0.803	+175	0.562	-7.06
750	40/5.34	3.18	1.54	52	0.779	+176	0.532	-5.69
915	32.8/4.46	3.04	1.60	51	0.772	+177	0.519	-4.32
1,500	20/2.81	2.42	1.77	49	0.761	+177	0.506	-3.66
2,450	12.2/1.76	1.70	2.21	47	0.754	+177	0.500	-3.88
3,000	10/1.45	1.61	2.26	46	0.751	+178	0.495	-3.20
5,000	6/0.89	0.778	3.92	44	0.749	+177	0.502	-4.95
5,800	5.17/0.775	0.720	4.73	43.3	0.746	+177	0.502	-4.29
8,000	3.75/0.578	0.413	7.65	40	0.744	+176	0.513	-6.65
10,000	3/0.464	0.343	10.3	39.9	0.743	+176	0.518	-5.95

TABLE VI

Characteristics of electromagnetic wave propagation in tissues of low water content (such as fat or bone) (Johnson and Guy, 1972)

Frequency ν (MHz)	Wavelength λ_o/λ (x 10^{-2} m)	Depth of penetration $1/\beta$ (x 10^{-2} m)	Conductivity σ (x 10^{-3} mho/m)	Dielectric constant K_e	Reflection coefficient at interface			
					Air/fat		Fat/muscle	
					r	ϕ	r	ϕ
100	300/10.6	60.4	19.1-75.0	7.45	0.511	+168	0.650	+172
200	150/59.7	39.2	25.8-94.2	5.95	0.458	+168	0.612	+172
300	100/41	31.1	31.6-107	5.7	0.438	+169	0.592	+172
433	69.3/28.8	26.2	37.9-118	5.6	0.427	+170	0.562	+173
750	40/16.8	23	49.8-138	5.6	0.415	+173	0.532	+174
915	32.8/13.7	17.7	55.6-147	5.6	0.417	+173	0.519	+176
1,500	20/8.41	13.0	70.8-171	5.6	0.412	+174	0.506	+176
2,450	12.2/5.21	11.2	96.4-213	5.5	0.406	+176	0.500	+176
3,000	10/4.75	9.74	110-234	5.5	0.406	+176	0.495	+177
5,000	6/2.63	6.67	162-309	5.5	0.393	+176	0.502	+175
5,800	5.17/2.29	5.24	186-338	5.05	0.388	+176	0.503	+176
8,000	3.75/1.73	4.61	255-431	4.7	0.371	+176	0.513	+173
10,000	3/1.41	3.39	324-549	4.5	0.363	+175	0.518	+174

depth of penetration (d). If the tissue layers have thicknesses less than d, then the reflected energy and standing wave patterns will be influenced by two factors, the wave impedances and the tissue thicknesses (Johnson and Guy, 1972).

(B) Biological dispersions

Biological systems are in fact neither conductors nor dielectrics, but may be a combination of both, depending upon the external influences acting on the system. For biological material in a constant electric field, the values of ε (or K_e) and σ are constant, dependent solely on the material. In an alternating electromagnetic field, ε and σ are determined by the frequency as well as by the chemical composition of the material. Above and below the microwave range, ε and σ are relatively constant for biological tissue, but within the microwave range, the values often vary drastically. This is because the reactivity of the various molecular elements that determine ε and σ change with the frequency of the electromagnetic wave.

The major reactive elements in most biological systems are molecular dipoles, membrane capacitors, and charged particles or ions. Membrane capacitor discharge as a result of an alternating field is one of the reactions of greatest magnitude in biological systems (Schwan, 1957). At low frequencies (<100 MHz), the response time of the cell membrane is such that the membrane can charge and discharge fully with the alternating field. This results in a high capacitive reactance, and conduction currents are not maintained within the cell. The tissue then has a low conductivity and a high dielectric constant. As the frequency of the alternating field increases, it becomes greater than

that of the cell membrane response time, and the membrane is no longer reactive. Now, the cell membrane no longer acts as a barrier to the flow of ions in and out of the cell, and the cell becomes electrically conducting. As the frequency increases, the electrical conductivity increases and the dielectric constant decreases. As a result, ε and σ vary inversely with respect to each other. This is shown in Figure 3.2. The frequency at which there is a decrease in ε and an increase in σ is referred to as the relaxation frequency for that element. As a result of this variation of ε and σ with frequency, it is possible for a material to behave as a conductor with respect to an electromagnetic field in one frequency range, act as a semi-conductor in another frequency range, and finally, behave as a dielectric in a third frequency range.

The loss tangent has been previously introduced in section 3.1.1 (B) as $\tan \delta = \sigma/\omega\varepsilon$. This is a useful index for describing the relative behavior of ε and σ. The medium behaves like a conductor when $\tan \delta >> 1$, i.e., the conduction loss greatly exceeds the dielectric loss. When $\tan \delta = 1$, the material acts like a semi-conductor and the magnitudes of the conduction loss and the dielectric loss are similar. The material behaves like a dielectric if $\tan \delta << 1$, i.e., the dielectric loss is much greater than the conduction loss. If $\tan \delta = 0$ ($\sigma = 0$), then the material is a perfect dielectric since the electromagnetic wave passes through the medium unattenuated. Values of the loss tangent in biological tissue are generally between 0.2 and 0.3 for 2450 MHz microwaves (Neuder, 1978).

There are three distinct frequency ranges in which ε and σ for

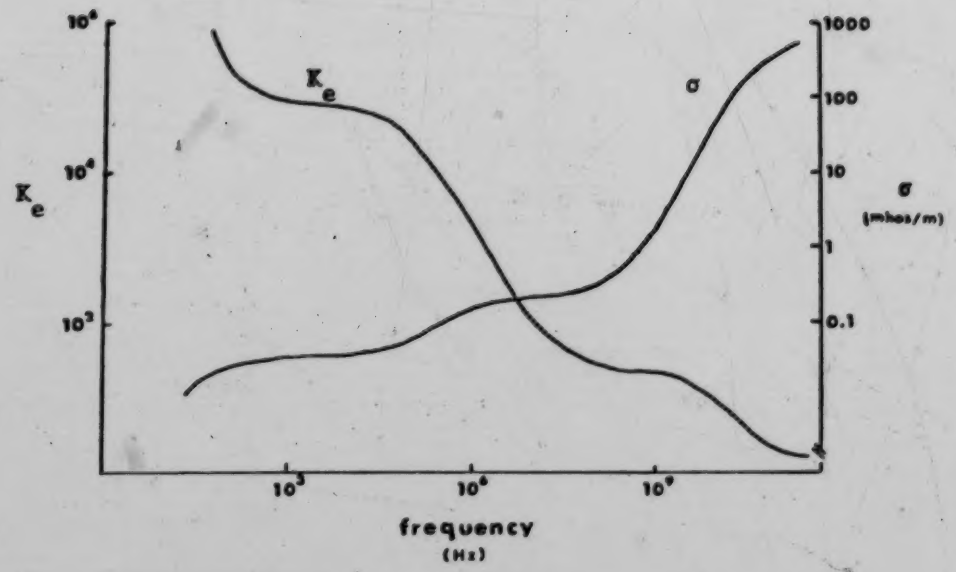


Fig. 3.2 Variation of K_e and σ with frequency for typical biological tissue with high water content (Grant, 1974).

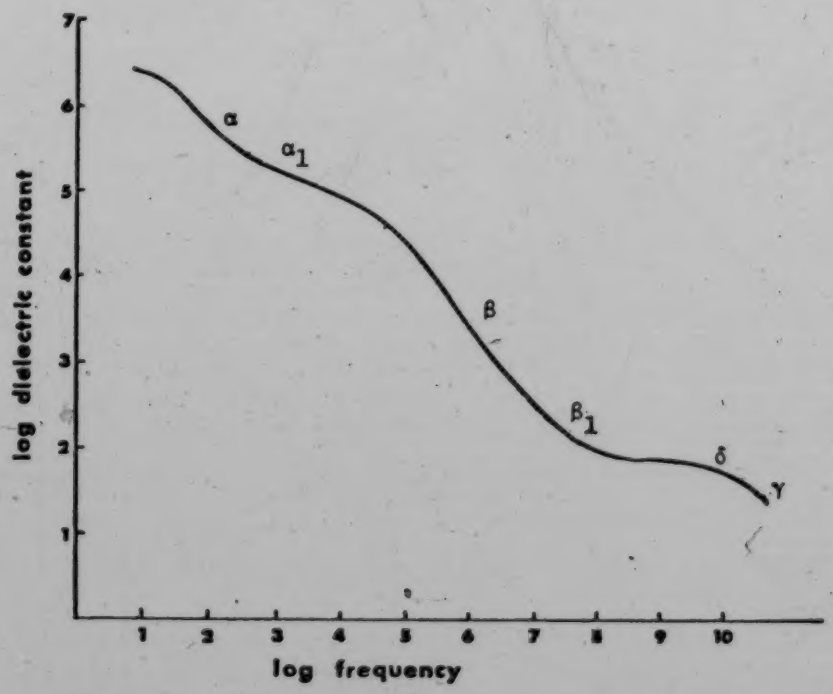


Fig. 3.3 The major and minor dispersions of muscle tissue (or any tissue with high water content) (Cleary, 1970).

tissue vary with the frequency of the microwave radiation. Regions in which there is a change in ε are referred to as dispersions. Figure 3.3 shows the three major dispersions for human muscle tissue which are arbitrarily designated α, β, and γ, along with several minor dispersions, α_1, β_1, and δ. Similar dispersion curves occur for any tissue with a high water content. In tissues with low water content, the curves are similar even though ε and σ are approximately an order of magnitude lower (Schwan, 1974).

At low frequencies, α dispersion occurs. This dispersion is the least clarified of all the major dispersions, although several mechanisms for its occurrence have been suggested. This dispersion may be due to the relaxation of charging and discharging processes on the cell membrane and any interconnected intracellular structures by the ionic environment around the cell's electrically charged surface (Schwan, 1957). Another suggested mechanism is the electrophoretic relaxation which results from an oscillatory movement of charged particles with the alternating field (Schwan, 1957). In this case, the cell membrane acts as an insulator and the low frequency currents can only flow in the extracellular medium. Biological cells and tissues exhibit α dispersion at frequencies less than 1 kHz. This dispersion has been observed in erythrocyte suspensions, yeast cell suspensions, squid axons, and muscle (Cleary, 1970). Since α dispersion has been found to occur in particles without membranes, this phenomenon does not necessarily involve the cell membrane.

The dispersion due to structural relaxation is referred to as β dispersion. A biological system is composed of many different structural units having different dielectric properties. If an electromagnetic

field is applied to the system, the current density in the system varies, and charge will accumulate at tissue interfaces until a constant current density is reached. The charge redistribution requires a certain amount of time depending on the structure, so relaxation effects will occur (Cleary, 1970). In a biological system there is a distribution of relaxation times due to variations in cell size and shape. Muscle, spleen, kidney, liver, and stomach wall tissue all show β dispersions in the radiofrequency range of 1-10 MHz. Thus, heterogeneous structure is responsible for β dispersion, such as polarization resulting from the charging of interfaces or cell membranes. Minor contributions to the β dispersion which cause the β_1 tail are due to the relaxation of tissue proteins and smaller subcellular structures (mitochondria, cell nuclei, organelles) (Schwan, 1957). These proteins and cellular structures possess a dipole moment which aligns the molecule with an electric field. This dielectric relaxation varies with the microwave frequency and occurs in the frequency range of 100 MHz to 1 GHz.

The γ dispersion in cell or tissue suspensions is due solely to the rotation of intracellular water molecules which possess a dipole moment. This occurs at a frequency of about 19 GHz. The fact that water exhibits a dispersion indicates that the level of hydration of a molecular or cellular system must be accounted for when results of in vitro experiments are applied to living systems. The relaxation frequency of bound water varies from 300 MHz on up, depending on how well bound the water is. The γ dispersion is characterized by an increase in conductivity in tissues with a high water content.

A minor dispersion, δ, occurs between the β and γ dispersions and

is caused in part by rotation of amino acids and charged side groups of proteins. It has been suggested that the relaxation of bound water, which occurs somewhere between 300 and 2000 MHz, may also contribute to this dispersion (Cleary, 1973).

3.1.3 Thermal and Nonthermal Microwave Effects

Thermal effects may involve either a general temperature elevation resulting from energy absorption or from a "specific" temperature elevation of a volume of matter above its environment (Schwan, 1968).

In contrast, nonthermal effects do not involve an increase in overall temperature. These effects include both "weak" and "strong" interactions with an electromagnetic field (Schwan, 1968). Dielectric saturation and orientation of unicellular particles occur at an electric field strength above 100 V/m. This corresponds to an incident power density of approximately 3 mW/cm^2. These effects are classified as "strong" nonthermal interactions.

Strong interactions have been shown to exist, but the presence of weak nonthermal interactions has never been completely substantiated.

(A) Thermal effects

The interaction of microwave radiation with a biological system will give rise to two types of processes: rotations of molecules with dipole moments according to the frequency of the electromagnetic field, and oscillations of free charges. Both of these processes will cause a loss of the electromagnetic field energy due to the viscosity and electric resistance of the medium. The loss of energy caused by rotation of dipoles in the medium is referred to as dielectric loss, while the loss of energy caused by the oscillations of free charges is called

conduction loss.

The physical basis for thermal effects is due to this energy exchange between the alternating electric field and free charges or dipoles. A molecule with a permanent dipole moment will experience a torque when placed in an electric field and attempt to assume an orientation in which the dipole moment is parallel to the direction of the applied electric field (Figure 3.4). In condensed states, such as liquids or solids, there will be a frictional resistance to the reorientation of the dipoles. There will be a loss of energy as heat due to the work needed to overcome the viscous damping forces of the medium surrounding the dipoles. This absorption of energy by matter is referred to as relaxation absorption. If the electric field is removed, the aligned dipoles return to their original states. This dipole relaxation, or dielectric relaxation, is associated with a characteristic time, the relaxation time τ. The relaxation time is a measure of the interval required for the order of the oriented dipoles to be reduced to 37% (1/e) of their initial value. Thus, τ is a parameter of the dipole structure and is independent of the applied field. The maximum energy absorption by the biological tissue occurs when the period of the oscillating electric field is of the same order of magnitude as the relaxation time τ. For example, water, which comprises a large portion of living tissue, has a relaxation time in the order of 10 picoseconds at room temperature. Thus, the maximum energy absorption in water occurs within a frequency range of 10^4 to 10^5 MHz (Marha et al., 1971).

The primary effect of the orientation and relaxation of dipoles with an alternating electric field is to cause a temperature increase

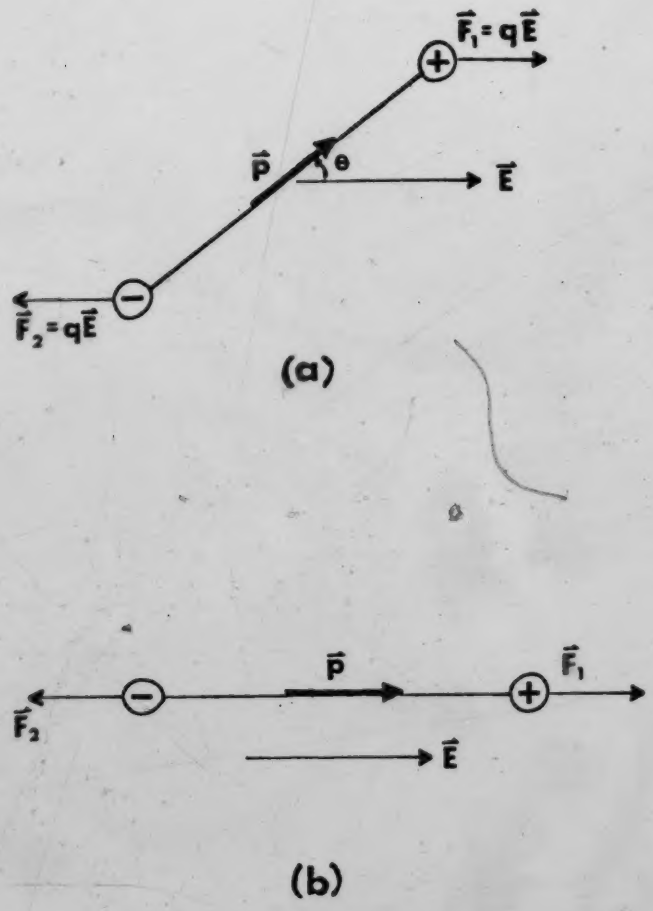


Fig. 3.4 Torque exerted on a dipole in a uniform electric field ($\vec{E}$). (a) The vector torque on the dipole is $\vec{\Gamma} = \vec{p} \times \vec{E}$. (b) The dipole is in equilibrium in a uniform field.

in the surrounding medium. This is referred to as primary heating or a thermal effect.

(B) Specific thermal effects

Specific thermal effects refer to primary heating of isolated structures which absorb microwave radiation independent of the surrounding tissue in which they are located. This selective heating is possible in biological material only for short periods of time (Schwan and Piersol, 1954). For longer periods of time, special irradiation techniques must be used. Transfer of heat via the circulatory system and through conduction and radiation dissipates heat rapidly from a site and thus minimizes selective heating. Schwan (1970) has concluded that selective heating can occur only when the structure is at least one millimeter in size. This is based on the rate of heat transfer as a function of particle size, and on the absorption characteristics of the biological material. Schwan has also concluded that during the initial transient temperature rise before thermal equilibrium is established, large groups of cells may be selectively heated. However, this heat will be dissipated very rapidly.

A direct consequence of microwave energy absorption is the induction of tissue specific temperature rises which lead to temperature differences between the various tissues as well as temperature differences within homogeneous material. This is referred to as primary heating whether it is a thermal or specific thermal effect, as compared with the distribution of this heat to other parts of the system. As mentioned before, the primary heat will be dissipated via conduction, radiation, and convection due to blood flow.

During the heating process due to microwave absorption, different types of tissues are heated to a greater extent than others. For example, surface layers of fat are heated more than deeper tissues for high frequency radiation, whereas for lower frequency electromagnetic radiation, the deeper tissues are heated to a greater extent. The energy absorption and heating patterns in a biological object will be complex since tissue layer thickness and depth varies, depending on the location in the body. Microwave heating may also be enhanced locally in areas adjacent to bone or tough fascial planes which act as reflecting surfaces. In general, microwave heating is greatest in tissues with a high water content such as muscle or skin.

(C) Nonthermal effects

The third type of effect, the athermal or nonthermal effect, does not involve a macroscopic increase in temperature of the irradiated system. As explained at the beginning of this section, these may be classified as either weak or strong interactions depending on the incident power density. The existence of nonthermal effects, while still not conclusively proven, is becoming evident as more experimental data is obtained.

One example of a nonthermal effect is the alignment of colloidal particles, unicellular organisms, leukocytes, or erythrocytes in saline with an electric field. The particles form chains parallel or perpendicular to the electric field when placed in an electromagnetic field with a frequency range from 1 to 100 MHz. This is referred to as the pearl chain effect (Herrick, 1958; Heller, 1959, Saito and Schwan, 1961; Presman, 1970) (Figure 3.5). This chain formation is due to the

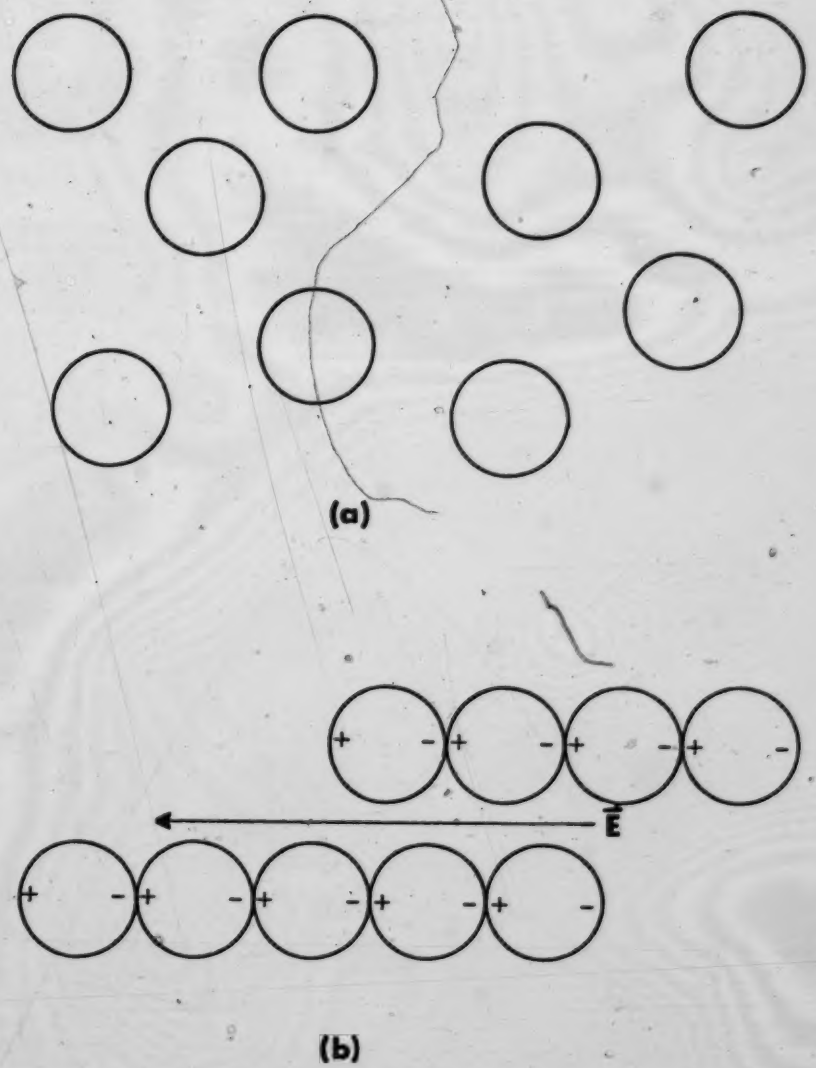


Fig. 3.5 The pearl chain effect. (a) Molecules are random when no electric field is present. (b) Molecules align themselves with the electric field ($\vec{E}$)

interaction between particles with dipole moments which were induced by the electromagnetic field. For each type of particle there is an optimum frequency range within which the effect occurs at a minimum field strength. The chain may be broken up by increased Brownian motion resulting from heat, by mechanical agitation, or by turning off the microwave field. Pearl chain formation of unicellular particles in biological systems occurs with an electric field strength on the order of 100 V/cm. This corresponds to an incident power density of approximately 3 mW/cm^2. Heating may become excessive at this field strength and mechanical agitation such as blood circulation may break up the chains, so it appears that it does not have a significant effect in larger organisms, such as man. Alignment of particles which results in very long chains of molecules has sometimes been referred to as "pseudo-macromolecule" formation.

A second nonthermal effect, dielectric saturation, may occur in solutions of proteins and other macromolecules due to intense electromagnetic fields with superhigh frequencies (Schwan, 1958). Such fields cause all the polar side chains of the macromolecules to become oriented in the direction of the electric lines of force. This can lead to breakage of hydrogen bonds and other secondary intra- and intermolecular bonds. This can also lead to alteration of the hydration zone which determines the solubility of the molecule. This could cause denaturation or coagulation of the molecules (Fleming et al., 1961).

Another possible nonthermal effect may be due to the action of Lorentz forces in alternating fields on ions in an electrolyte (Heinmets and Hershman, 1961). If an electrolyte solution is exposed to mutually

perpendicular, in-phase electric and magnetic fields, then the electric field averaged over all time has no effect on the ions. Lorentz forces may cause the positive and negative ions to move perpendicular to the electric lines of force. Heinmets and Hershman (1961) point out that the effect depends on the sum of the ionic abilities, and that the effect can be produced by an electromagnetic wave propagating in a medium.

3.1.4 Microwave Effects at the Molecular Level

High intensity microwave fields cause an increase in the average molecular kinetic energy of a material leading to denaturation or irreversible thermal damage. This is not true of low intensity microwave fields which have been defined as interacting with specific molecules or receptors in the material (Cleary, 1970). These receptors would absorb the microwave energy for a period of time and then redistribute it as thermal energy to the rest of the biological system. Some functional alteration in the receptor, most likely reversible in nature, would occur as a result of the energy absorption. The nature of this alteration would depend both on the relaxation frequency of the molecule and on the frequency of radiation. Due to the low intensity of the radiation, the functional alteration would have minor biological consequences (Cleary, 1977).

The controversy that presently exists regarding the existence of nonthermal microwave effects can be resolved by investigating the mechanism of interaction of low intensity microwave fields with a biological system. Many suggestions have been made for possible molecular effects caused by low intensity microwave fields, such as an alteration in the structure of bound water surrounding biological macromolecules,

and disruption of hydrogen bonds in proteins, nucleic acids, and other macromolecules (Cleary, 1973).

A change in the energy level of an orbital electron is associated with the absorption of a photon in the visible, ultraviolet, or x-ray region of the electromagnetic spectrum. Infrared radiation is associated with smaller transitions in energy which involve alterations of interatomic bonds in a molecule. This causes rotational and vibrational excitation. One quantum of microwave radiation has an energy of 4×10^{-4} to 1.2×10^{-6} eV, which is too low to cause any of the above energy transitions independent of the intensity of the radiation.

The activation energies (energy required to produce an alteration in the molecule) for various molecular effects are tabulated in Table VII (Cleary, 1973). From the activation energies it is evident that microwaves cannot cause any of the molecular effects unless there is a frequency-specific effect, or possible cumulative effect.

There are a number of different types of intra- and inter-molecular bonds in biological systems. Each of these bonds corresponds to different degrees of atomic interactions, and thus may be more or less susceptible to low intensity microwave fields. Too much energy is required to disrupt a covalent bond, which is responsible for the primary structure of the molecule, to be broken by a low intensity microwave field. There are, however, a number of secondary bonds, such as hydrogen bonds, hydrophobic interactions, and London-van der Waals forces, which are responsible for the secondary and tertiary structure of the molecule. These bonding forces determine the conformation and the stability of the molecule or molecular complexes. Since the conformation of a molecule

TABLE VII

Activation energy and radiation parameters for various molecular microwave effects in biological systems (Cleary, 1973)

Effect	Activation Energy		Radiation Parameters	
	kcal/mole	eV	Frequency (GHz)	Wavelength (μm)
Microwave radiation	2.7×10^{-6}-0.03	1.2×10^{-7}-1.2×10^{-3}	0.03-300	10^{7}-10^{3}
*Disruption of bound water	12.9	0.56	1.4×10^{5}	2.14
Thermal or Brownian motion 30° C	0.60	0.026	6.3×10^{3}	47.6
Rotation of polar protein molecules	0.92-9.2	0.04-0.4	9.7×10^{3}-9.7×10^{4}	30.9-3.1
Hydrogen bond disruption	1.8-4.6	0.08-0.2	1.9×10^{4}-4.8×10^{4}	15.8-6.25
Reversible conformational changes in protein molecules	9.2	0.4	9.7×10^{4}	3.1
*London-van der Waals interactions	23	1	2.4×10^{5}	1.25
Proton tunneling	16.1	0.7	1.71×10^{5}	1.76
Semi-conduction	23-69	1-3	2.4×10^{5}-7.25×10^{5}	1.2-0.41
Covalent bond disruption	50-115	2.2-5	5.28×10^{5}-1.21×10^{6}	0.57-0.25
Charge transfer interaction	138-169	6-7.34	1.45×10^{6}-1.76×10^{6}	0.2-.17
Ionization	230	10	2.4×10^{6}	0.12

*These effects are summations of many interactions.

specifies its function, these steric relationships are important. Most of these secondary bonding forces have a much lower activation energy than that of covalent bonds and thus may be potentially more sensitive to disruption by low intensity microwave fields. Determination of whether this occurs is complicated by the fact that the bonding forces responsible for secondary and tertiary structure are cooperative in nature. The rate of disruption or reformation of bonds depends on how many interactions have already occurred. An induced stress, such as an alternating electromagnetic field, could disrupt this weak secondary bonding, causing reversible or partial denaturation of molecules (Cleary, 1977). This effect would be difficult to detect since the molecule could quickly refold.

Bound water is defined as water molecules which are held in non-random orientations at or near the surface of a macromolecule in a hydration layer that is one to several molecules thick (Cleary, 1977). Hydrogen bonding between the water molecules and charged side chains of macromolecules holds the bound water in place. If low intensity microwaves disrupt or alter the state of bound water at the surface of a macromolecule, conformational and thus functional states of the biomolecule could be affected. Studies of the dielectric dispersion of aqueous protein solutions have indicated the existence of protein bound water (Cleary, 1977), but the energy required to disrupt bound water has not yet been determined. The activation energy for bound water can be extrapolated from that of water molecules in solution. Studies show that for water molecules in solution, the energy required for reorientation of water molecules depends on the number of hydrogen bonds between

molecules. For bound water, the symmetry of the bonding and the distance between the water molecules and the macromolecule would determine the activation energy. The activation energy for disruption of bound water has been estimated at 0.56 eV, which corresponds to photons in the infrared region (Ling, 1971). Whether low intensity microwave radiation can alter the state of water which is bound to macromolecules still remains to be proven.

Some of the possible microwave interactions at the molecular level, leading to both thermal and nonthermal effects are shown in Figure 3.6.

3.2 Effects of Microwaves on Biological Macromolecules

3.2.1 Proteins

Low intensity microwave effects on mammalian cells are generally reversible, with varying time constants for the relaxation of the system to its unperturbed state (Cleary, 1970). It has been suggested that low intensity nonthermal effects may be due to subtle conformational changes in protein molecules. These changes are often hard to detect since the time constant for reversion to an unperturbed state is proportional to the field intensity. Thus, the system must be observed during or immediately after exposure to microwave radiation.

Many experiments have been conducted using a wide assortment of proteins, with equally diverse radiation parameters and techniques for the detection of molecular effects (Schwan, 1958; Vogelhut, 1960; Prausnitz et al., 1961; Takashima, 1966; Yeargers et al., 1975; Cleary, 1977). Some of the suggested mechanisms for microwave interactions with proteins and enzymes will be summarized in this section.

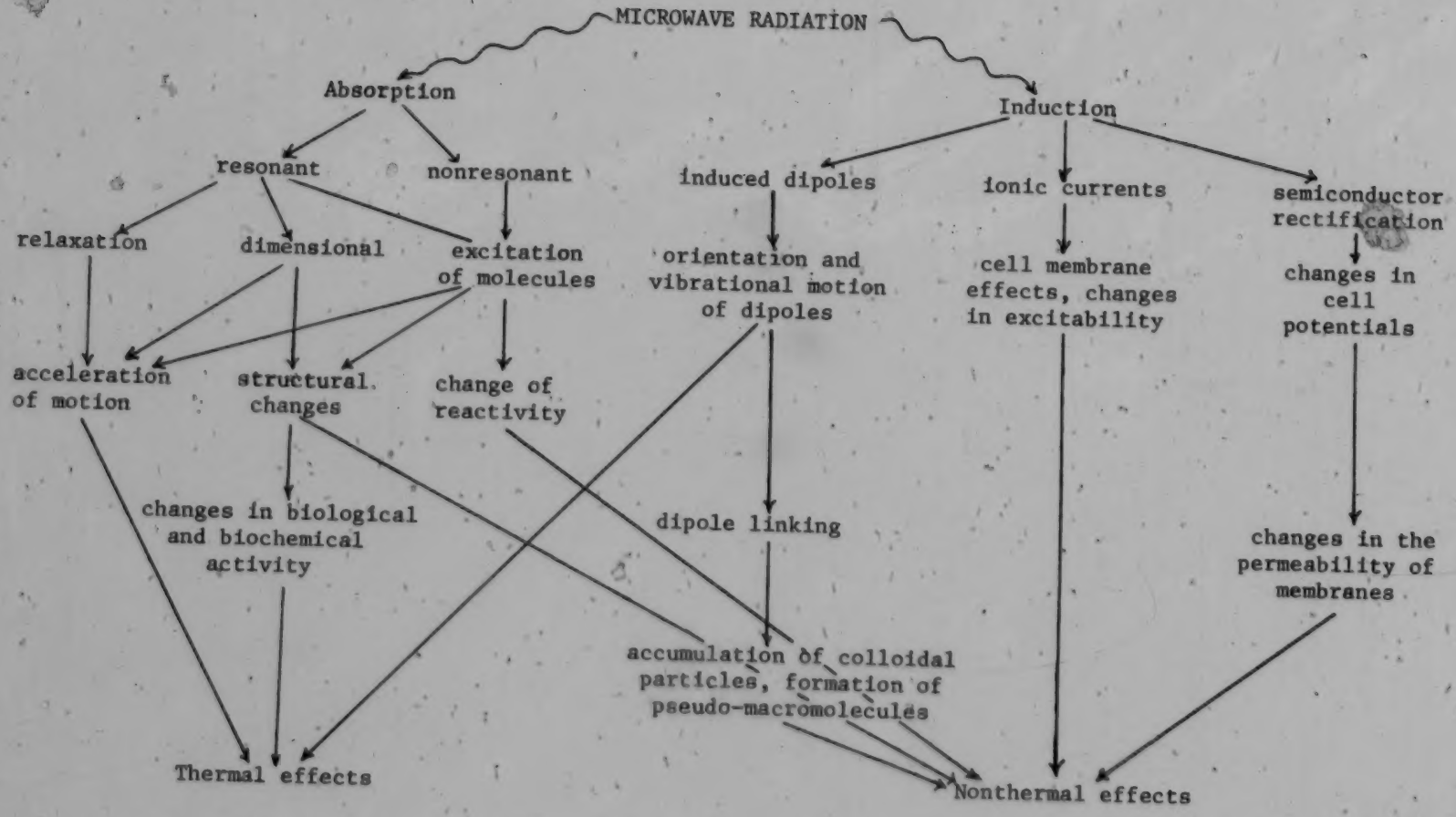


Fig. 3.6 Microwave interaction with a biological system (Marha, Musil, and Tuha, 1971).

Enzymes consist of many amino acid residues joined together in a twisted helix which is held together by weak hydrogen bonds. This bonding determines the conformation or three-dimensional structure of the enzyme and thus the specific reaction the enzyme catalyzes. From Table VII, it can be seen that it requires as little as 0.08 eV energy to break a hydrogen bond. Enzymes are denatured by breaking the hydrogen bonds which give the biomolecule its conformation and thus its catalytic properties. Hydrogen bonds may be broken by thermal energy absorption, direct interaction with an alternating electric field, and ultraviolet light.

It has been postulated that oxidation-reduction reactions which require enzyme catalysis may involve the attachment of enzyme to substrate by two hydrogen bonds which serve as the conduction pathway for electrons. When the reaction is completed, the hydrogen bonds dissociate. The enzyme is then free to interact with other substrate molecules (Cleary, 1977). If an induced electromagnetic field interfered with this process, enzyme function will be inhibited. The biological consequence will be manifested as an interference with electron transport, and thus cellular respiration (Cleary, 1977).

Two mechanisms which can cause microwave energy absorption by an enzyme in solution will be discussed. The microwave field can interact directly with the enzyme molecule by a relaxation phenomenon (Yeargers et al., 1975). However, most enzyme molecules have an extended geometry and a large dipole moment and therefore relaxation is not likely to be a significant mode of microwave energy absorption. In fact, the principal relaxation frequencies for some enzymes (lysozyme, trypsin) have been

found to be about 3 MHz (Yeargers et al., 1975). However, this does not mean that smaller subunits of the enzyme will not interact directly with a microwave field by a relaxation process. Microwave energy may also be absorbed by solvent molecules and the resultant thermal energy transferred to enzyme molecules by molecular collisions. Many solvents (for example, phosphate buffered aqueous solutions) are highly lossy at microwave frequencies and thus strongly absorb microwave energy. This absorbed energy, converted to heat, can denature the enzyme molecules in solution (Yeargers et al., 1975).

Yeargers et al. (1975) found that there is no discernible difference in enzyme performance whether heated conventionally or by microwaves. In both cases heating caused denaturation of the enzyme to approximately the same degree depending on the temperature.

When an enzyme solution (alcohol dehydrogenase, bovine serum albumin, or bovine hemoglobin) was exposed to a low intensity radio-frequency field which did not involve an increase in solution temperature, no decrease in enzyme activity was observed (Takashima, 1966). This means that low intensity radiofrequency waves do not have a nonthermal effect on the enzyme solutions, or that the effect was too small to affect enzymatic activity.

The possibility of resonance absorption of electromagnetic fields by protein molecules has been investigated (Vogelhut, 1960; Prausnitz et al., 1961). In protein molecules, the number of polarized side groups usually exceeds the number of protons bound with them (except strongly acidic solutions) so that many configurations of proton distribution in the molecule exist with small free energy differences (Vogelhut, 1960).

These dipole-dipole interactions are due to fluctuations in proton distribution and may be responsible for the absorption of a quantum of microwaves at a frequency of 10 GHz. This resonance effect of electromagnetic fields on proton distributions in enzyme molecules can lead to a change in the rate of formation of enzyme-substrate complexes (Vogelhut, 1960; Prausnitz et al., 1961). Exposure to a SHF field with a particular resonant frequency in the range 8.2-12.4 GHz was found to alter the optical density of lysozyme and the rate of its enzyme-substrate reaction (Vogelhut, 1960). Chernavskii et al. (1967) determined that the frequency of vibrations for lysozyme molecules due to their elastic deformation is 10-50 GHz. These conformational vibrations of protein molecules are due to folding, twisting or compression of the polypeptide chains and lead to displacement of the electric charges on the surfaces of the molecules (Shnol', 1965, 1967). This might tie in with the resonance effect for lysozyme found at approximately 10 GHz, since electromagnetic fields can interact with the vibrations.

Resonance absorption of electromagnetic fields of superhigh frequencies in protein molecules or other macromolecules may involve intramolecular processes such as translational shifts of hydroxyl groups from one hydrogen-bonded position to another and rotation of intramolecular structures about C-C bonds (Barber, 1962).

There is a characteristic frequency at which there is a maximum energy transfer from the microwave field to rotating polar biomolecules and subsequently to the biological system. This energy transfer is referred to as dielectric relaxation, as previously discussed. The dependence on frequency is called dielectric dispersion. Aqueous protein solutions exhibit two principal dispersions, one at 1-10 MHz (β) and the

other at approximately 20 GHz due to dipole relaxation of free water molecules (δ). Small peptides and amino acids exhibit principal dispersions in the 0.3-3 GHz region.

Microwave or radiofrequency fields which interact with polar protein molecules in the frequency range 0.1-20 GHz cause rotation of the molecule as a whole, rotation of bound water molecules, or rotation of polar side chains or segments of a molecule. This can cause changes in the conformation of a protein which can result in changes in function. However, the power densities required for in vivo molecular rotations are most likely in excess of 10 mW/cm^2 and thus cannot be classified as nonthermal interactions.

Dielectric saturation, which can occur in solutions of proteins and other biological macromolecules, is caused by intense electromagnetic fields with superhigh frequencies (Schwan, 1958). These fields can cause polarized side chains to align themselves with the electric lines of force. This can lead to breakage of hydrogen bonds and to alteration of the hydration zone (Schwan, 1958). These effects can cause denaturation or coagulation of molecules (Fleming et al., 1961).

Studies have shown that an alternating electric field with an intensity of approximately 10^4 V/cm can exert an influence on the elasticity of protein chains (transition between a long and short chain) which results in muscle contraction (Hill, 1958).

3.2.2 Nucleic Acids

Very little is known about the effect of microwave radiation on nucleic acids. High intensity microwaves may cause heating of cells or tissues which can cause denaturation or coagulation of proteins and

nucleic acids. There have been genetic effects associated with the interaction of both low and high intensity microwave fields with biological systems. These effects include an increase in the frequency of dominant mutations, chromosome aberrations, and cessation of mitosis (Presman, 1970). This implies that some alteration has occurred either in the nucleic acid structure or some intracellular process associated with the function of nucleic acids.

Dipole-dipole transitions in protein molecules have been discussed previously. If these transitions, which are due to fluctuations of the charge distribution on the molecule, occur in DNA molecules, then a resonance effect could be expected to occur. Disturbances of the interactions between DNA molecules or between DNA and RNA molecules could also occur as a result of interaction with a microwave field.

DNA depends on hydrogen bonding for the specificity of the genetic code. If a proton involved in a DNA hydrogen bond shifts from one potential minimum to the other by the quantum mechanical tunneling effect, the other proton will move in the opposite direction. This maintains electronic equilibrium although the altered base pair may cause a point mutation at the next replication cycle of the DNA. The energy required for shift of a proton to another base pair in DNA in solution is 0.7 eV. There is indirect evidence to suggest that spontaneous mutations in DNA occur as a result of double-proton tunneling in the molecule, but not that it may be caused by any microwave or radiofrequency interactions. Mutations due to proton tunneling would be expected to be caused by thermal mechanisms as the radiation frequency associated with this effect lies in the infrared region of the spectrum (Cleary, 1973).

DNA exhibits dielectric dispersion in the 1 kHz frequency range (Takashima, 1963). Thus, it absorbs energy in the radiofrequency portion of the electromagnetic spectrum. DNA in vitro exposed to radiofrequency fields in the frequency ranges of 10 Hz to 10 kHz and 100 kHz to 10 MHz did not show any structural change (Takashima, 1966). In order to cause a structural change, the energy absorbed must exceed the bond dissociation energy times the number of bonds which need to be broken to affect a change. This occurs with ultraviolet light, with energy of about 10 eV at a wavelength of 10^3 Å, compared with the energy of 1 MHz radio-frequency waves which is approximately 10^{-9} eV.

3.3 Whole Body Microwave Absorption

The loss of energy by microwaves and the subsequent absorption by biological tissue depends on the frequency of the radiation and the electric and magnetic properties of the tissue. When dealing with a whole biological object, many factors must be taken into account. Factors which affect whole body microwave energy absorption are:

1. Frequency (wavelength) of the radiation.
2. Modulation of the radiation; pulsed or continuous.
3. Incident power density.
4. Duration of the exposure.
5. Size, dimensions, and orientation of the object in space relative to the direction of propagation and plane of polarization of the incident microwaves.
6. Size, dimensions, and spatial orientation of any electrical heterogenities in the object, and the thickness of tissue layers with different electrical properties.

7. Biochemical properties of the object (electric and magnetic properties).
8. Texture of skin; presence or absence of fur, hair, or clothing.
9. Ratio of surface area to body mass.
10. Thermoregulatory capacity of the organism.

To conclude this chapter, a review of several whole-body microwave effects is given.

A living system, regardless of complexity, is self-regulatory and homeostatic. A self-regulatory system controls and regulates its reactions to adapt to changes in its environment. Homeostatis is the maintenance of system equilibrium and the mechanism by which this constancy is maintained. This includes regulations of such variables as temperature, water content, pH, and salt, ion, and nutrient concentrations.

A living system may be studied at any level of organization; molecular, subcellular, cellular, at the level of tissues or organs, or in terms of the whole organism. Whichever viewpoint is considered, any interaction of any part of the system with microwaves which causes a perturbation of the system, will lead to a manifestation of the interaction as a secondary effect. In other words, the system reacts to the primary interaction with microwaves in such a way as to attempt to maintain its internal equilibrium.

Cleary (1973) has formulated a partial listing of biological effects which have been attributed to interaction of alternating electromagnetic fields in the microwave and radiofrequency range (Table VIII). Cleary points out that the diversity of these findings raises the question of how microwaves and radiofrequency waves can interact with

TABLE VIII

Partial Listing of Reported Microwave and Radiofrequency Effects in Biological Systems (Cleary, 1973).

I. In vivo effects

A. In humans

1. Thermal responses: hyperthermia; cataracts; cardiovascular effects (lability of pulse and blood pressure; bradycardia and tachycardia); heart enlargement; EEG changes; increased thyroid activity; alterations in serum proteins; respiratory changes; lenticular opacities; increased incidence of Down's syndrome; altered sex ratio; alterations in albumin-globulin ratio; histamine elevations in serum; disruption of endocrine-humeral processes; disruption of sexual potency.

2. Nonthermal responses: auditory nerve responses; neurological effects (hallucination, syncope, adynamia, other manifestations of "diencephalic" syndrome); fatigability; headaches; sleepiness; irritability; loss of appetite; memory difficulties; decrease in olfactory sensation; hair loss; unstable mood; hypochondriasis; anxiety; reduction in auditory sensitivity.

B. In experimental animals

1. Thermal responses: hemoconcentration; hemodilution; pupillary dilation; hyperthermia; burns; vascular hypertension; hemorrhage; testicular effects (atrophy, edema, enlargement, fibrosis, necrosis of seminiferous tubule, reduced spermatogenesis); decrease in eosinophils and lymphocytes; chromosomal aberrations.

2. Nonthermal responses: mutations (sex-linked, autosomal, dominant, recessive, sex-linked recessive lethals); neurological (CNS) effects.

II. In vitro effects

A. Thermal responses: mitotic arrest in cell cultures; bactericidal effects; alteration of plant growth; chromosomal aberrations; plant tumour growth arrest; excitation of frog muscle and heart preparations.

B. Nonthermal responses: resonance absorption in methyl palmitate; pearl chain formation (blood cells and unicellular organisms); neuronal interactions; enzyme inactivation; orientational effects in microorganisms; dielectric dispersion of cells and biomolecules.

matter to produce such a wide variety of biological effects. It is doubtful that all of the effects in Table VIII can be attributed to microwaves or radiofrequency waves. In many cases, the system variables are not well-defined; there is no list of the frequencies, power densities, physical parameters of the system, or conditions of exposure for each for the purported effects. However, with more experimental data, better technique, and more accurate methods of determining absorbed dose, this list will be refined.

Secondary effects of microwave interaction with biological tissue may be classified in terms of early or late effects. Early effects may occur immediately or shortly after exposure to microwaves. When a whole body is exposed to microwaves, the radiation is absorbed and converted to heat. This heat must be dissipated by various body mechanisms such as conduction, convection, radiation, or evaporation of sweat. Mechanisms which operate to distribute the primary heat are classified as early effects and may be manifested as a generalized or local rise in temperature, or hyperthermia. If the thermoregulatory mechanisms of the system can dissipate the primary heat as soon as microwave energy is absorbed, then no temperature rise will occur. However, a secondary thermal effect still does exist in the activation of various thermoregulatory mechanisms such as dilation of blood vessels and increased blood flow. Secondary metabolic effects related to changes in the blood flow may occur. Local thermal injury to blood vessels leading to a change in the metabolic rate may also occur. These injuries are due to a high level of primary heating caused by unequal energy absorption. The result of this may be an impairment of heat transfer through the circulatory system.

Unequal energy absorption which leads to unequal deep body heating is not an uncommon biological effect. Several parameters determine the distribution of primary heat, one of which is the internal and external geometry of the complex biological object. The distribution of primary heat, and thus thermal injuries, will vary depending on the size and shape of the organism, and for microwaves with a given wavelength, power density, and duration.

The magnitude of any temperature rise thus depends on the difference between heat generation and heat loss. When part of the body is exposed to microwaves, the blood flow carries heat to cooler parts of the body thereby stabilizing the temperature of the exposed part of the body. In general, the mechanisms of thermal regulation available to a species and the efficiency of these mechanisms will affect the degree of damage caused by production of a specific amount of heat within a body.

Severe high body temperature may cause many biological effects. These include denaturation of protein, tissue destruction, fever, decrease in enzyme activity, liberation of toxins, increased permeability of cell membranes, and impairment of neuron function. At 40° C, the central nervous system cannot function normally; prostration, coma, and death may result. With an increase of 10° C, the rate of chemical reactions may be doubled (Herrick and Krusen, 1953). In general, the higher the temperature increase, the greater the damage to critical organs and the body.

People exposed to high intensity microwaves on the limbs or forehead have an awareness of microwave exposure if the duration is sufficient. This is expressed as pain and/or warmth. These symptoms are

manifested when the energy absorbed in a critical thickness of superficial tissue exceeds a certain value. A thermal pain sensation is manifested when end organs 1.5 mm below the skin surface reach a temperature of approximately 46° C (Cook, 1952).

Late secondary effects may be manifested as genetic changes, rearrangements within molecules or subcellular components, gross structural aberrations, neural effects, or lenticular effects, among others. Several of these secondary effects of microwave interaction with biological tissue will be discussed.

Lenticular effects refer to cataracts or opacities of the lens caused by microwaves. The lens of the eye is designed to focus visible light on the retina. Microwaves, however, are not focused on the retina, but within the eye itself (Cleary, 1970). The focusing of microwaves in the eye causes a "hot spot" which can cause the lens protein to coagulate, or form an opacity or cataract (Michaelson, 1972). "Hot spots" occur partly because the eye lacks blood vessels to distribute the primary heat. The eye also consists of post-mitotic cells which are unable to repair damage as well as cells undergoing replication. Lens cataracts have occurred in bovine eyes with various frequency microwaves at 80 mW/cm^2 incident power density, repeated for 10 days. However, the same frequency microwaves at 80 mW/cm^2 for a one hour exposure had no effect (Michaelson, 1972).

Specific effects of microwaves are due to stimulation of special microwave or radiofrequency receptors within an organism under certain conditions and are not induced by other means (Presman, 1970).

Microwaves have been observed to cause an auditory response in

man (Frey, 1962). This has occurred with a microwave power density as low as 100 $\mu W/cm^2$, with an upper limit of 10 mW/cm^2. This implies that this is a nonthermal effect. The nature of the response has been described by various people as a buzz, ticking, knocking, humming or a whistle depending on the pulse width and pulse repetition rate, generally 600-2500 pulses/second, with a duration of 1-6 μsecond (Frey, 1962). The sound appeared to be localized within or behind the head. The greatest sensitivity to the sound was in the frequency range from 300 to 1200 MHz (100-25 cm wavelength) and varied depending on the modulation and peak power density. Frey characterized this as a direct auditory nerve response, while Sommer and von Gierke (1964) thought it was most likely due to stimulation of the cochlea through air or bone conduction of the electromagnetic field forces.

Reversible effects are expressed in the form of functional changes in experimental animals or humans and usually occur after long-term irradiation with microwaves at nonthermal intensities (Presman, 1970). These effects may become irreversible if the duration of exposure is sufficient. Reversible effects are due to an accumulation of functional changes in various organs or reflexogenic areas. The most significant reversible effects were vagotonic reactions of the cardiovascular system: bradycardia; hypotension; reductions in CNS activity (such as reduction of sensitivity to sound, olfactory stimulation, and inhibition of conditioned-reflex activity); and blood changes (such as alterations in the white blood cell count, albumin-globulin ratio, and histamine level). The most pronounced vagotonic reactions in man were due to millimeter wavelength radiation which was completely absorbed in the skin. This led

to the suggestion that vagotonic reactions were due to the effects of microwave radiation on neural skin receptors since the energy was superficially absorbed (Presman, 1970).

The degree and nature of expression of reversible effects depend on the duration and modulation of the microwaves and on the portion of the body irradiated. Once the irradiation of the body was stopped, normalization of functions occurred in a period of time from a few days to several weeks.

Soviet researchers have reported that the central nervous system is the organ system which is most sensitive to low intensity microwaves of long duration (Presman, 1965). Some of the reported effects in occupationally exposed workers include bradycardia, hypotension, a decrease in olfactory sensitivity, increase in blood histamine content, sleepiness, irritability, fatigue, and headaches. The lack of statistics and detail of physical conditions of exposure are not sufficient to enable determination of whether the CNS effects are nonthermal or due to low levels of thermal stimulation of specific receptors.

One final secondary effect worth noting is the possibility of microwave exposure affecting neural response. This has long been a controversial subject. MacGregor (1970) has theoretically proven that microwaves could affect neural excitation, but it has also been proven using the same substrate, that microwaves cannot affect nervous activity (Schwan, 1974). Western researchers, exposing biological systems to low frequency, high intensity microwaves of short duration, have proven that the latter is true (McAfee, 1962). Soviet and Eastern European researchers have exposed biological systems to high frequency, low intensity microwaves for long periods of time and proven that the former is true (Presman,

1965; Gordon et al., 1973). The disputed segment of the microwave range has been studied and it was found that there is a significant depression in conduction velocity between 2.5 GHz and 8 GHz, but no significant effect on neural transmission below about 2.5 GHz (Deficis et al., 1973). Lords et al. (1973) postulated that the synapse is a site of action for microwave effects. This is supported by evidence showing that if synapses are exposed to microwaves at low intensity for a length of time, there is a loss of transmission efficiency (Seaman, 1973). The growing number of experiments on neural effects may soon resolve the existing controversy.

CHAPTER IV

RADIOBIOLOGY OF CULTURED MAMMALIAN CELLS

4.1 Cells in Culture

The basic unit of any living organism is the cell, which may exist independently or with other groups of cells to form organisms of varying complexity. Multicellular organisms consist of a large number of different types of cells which develop from a single embryonic cell and perform a wide variety of functions. By their nature, cells comprise a heterogeneous group; each cell population has differences not only in structure but in function.

Cell populations as a whole are heterogeneous; however, there are several characteristics common to all. Cells may exist either in steady state or dynamic populations. Cells in a steady state population maintain a constant number of cells, even though some cells die and some reproduce. Dynamic populations change in total number of cells and may be either growing or dying.

Cell cultures have been widely used in cancer research, as cultured cells often have undergone similar changes in metabolism and dedifferentiation of function as malignant cells. It must be kept in mind, however, that cells in vitro are inevitably altered after removal from a living organism. Cells in vitro may be used to study metabolism and cell behavior, structure and function, and the interaction of cells with the environment. Cell reproduction in vitro is widely used for measuring the effects of alterations in the physical and chemical environment. It is

not possible, however, to determine from cell cultures how the cells interact together within a tissue or with other tissues in a specific bodily function.

In an organism, morphological and metabolic characteristics can be used to identify a large number of different cell types. When cells are cultured, many of the recognizable differences disappear or the cell may dedifferentiate.

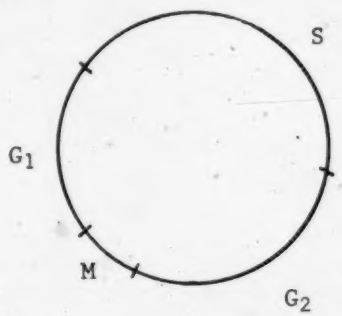
Cells which have been removed from an organism and suspended in a tissue culture medium may be resuspended in fresh medium to provide an ongoing cell culture (Paul, 1975)[1]. If the cells can be repeatedly sub-cultured, they are referred to as a primary cell line. These cell lines often cease to proliferate after a length of time which is specific for a cell population. Some cell lines may be cultured indefinitely in vitro, and these are referred to as established cell lines. These cell lines sometimes arise as a few colonies of rapidly growing cells in a slower-growing primary cell line. In some cases, there may be a gradual transition from a primary cell line; more often, an alteration occurs leading to an aneuploid chromosome number and a fast doubling time (12-20 hours). All established cell lines have similar nutritional requirements, grow in cultures to a relatively high density, and rarely show a specialized function. This distinguishes them from primary cell lines which preserve many characteristics of the cells from which they were derived.

4.1.1 The Cell Cycle and Growth Cycle

Proliferating cells in vitro follow a cell cycle. In eukaryotic cells, this cell cycle has four stages. The stage in which protein and

RNA synthesis begins (first gap or G_1) varies greatly in length. DNA synthesis (S) begins at the end of G_1 and lasts from 4 to 6 hours in mammalian cells (Paul, 1975). This is followed by the second gap (G_2), which usually lasts about 4 hours. During this time, the macromolecular synthesis is complete and the cell has accumulated sufficient energy to divide. The division of the cell into two daughter cells occurs during mitosis (M) and generally lasts 30-60 minutes in mammalian cells (Paul, 1975).

Cells in culture usually maintain a similar cell cycle, but are asynchronous and replicate at different times.



Cells in vitro may be synchronized by the addition of various chemical substances which block the cell cycle at a particular point, often in metaphase. This allows cells to progress through the cycle up to that point. When the chemical substance is removed from the cell culture, a large number of cells undergo mitosis at the same time. This synchrony usually lasts only one or two cell cycles.

Cells may be grown in vitro by a process referred to as tissue culture, or more strictly speaking, cell culture. Cells obtained from tissues in an organism may be grown in suspension in a liquid medium (Paul, 1975). Cells which are resuspended in medium will not grow for a

time while the cells adapt to the environment. This is referred to as the lag phase. Once the cells begin to divide rapidly, they do so at an exponential rate, which gives rise to the term logarithmic or log phase. Cell growth is characterized by the mean generation time (MGT) or the time it takes a cell population to double in number. This is generally slightly longer than the time it takes a cell to complete one cycle due to a small percentage of non-viable cells. The cells will actively metabolize and divide until the medium is depleted of necessary nutrients, the cells reach a critical inhibiting cell density, or the accumulation of cytotoxins inhibits cell growth. The cells stop replicating at this point and the number of non-viable cells increases to a maximum. This is referred to as the stationary phase. The last stage of cell growth in vitro shows a decrease in cell number, as cells begin to die. Cells which are to be carried in vitro must be resuspended in fresh medium every two to three days. The resuspension is usually done when the cells are in late log phase. This minimizes the lag phase in the subsequent generation and the cells begin dividing optimally soon after resuspension.

4.1.2 Growth Curves

During the logarithmic phase, cell growth may be described by the equation:

$$N = N_o e^{kt}$$

where N = total number of cells at time t

N_o = initial number of cells

kt = generation number

Growth curves may be plotted on semi-logarithmic paper for cells which are grown in suspension cultures. The four different phases of growth for a mammalian cell population are indicated in Figure 4.1. This growth curve is specific for lymphocytic mouse leukemia cells, line L1210. The mean generation time for the cells in log phase is 12.8 hours. The calculation for MGT is outlined in Appendix I.

4.1.3 Cell Death

Mammalian cells which are exposed to ionizing radiation may incur a variety of lethal or sublethal damages. At the cellular level, the criterion for a lethal event is cell killing. In mammalian cells, exposure to high doses of ionizing radiation in the kilorad range can cause a rapid cessation of cellular metabolism and disintegration of cells. This is referred to as non-mitotic death and occurs in non-dividing or slowly dividing cells. Mammalian cells which are rapidly dividing may be killed by exposure to much lower doses of ionizing radiation. This killing does not occur by cellular disintegration but by inhibition of the cell's ability to divide and proliferate and is called reproductive death. After irradiation, cells which are killed may not be able to reproduce at all, or if they are capable of post-irradiation reproduction they will produce sterile progeny. These cells may appear normal in all aspects but are technically defined as having been killed. Cells may also degenerate after doses of a few hundred rads without any attempt to replicate. Some cells may continue to replicate, but fail to divide, and thus form "giant" cells. Reproductive death, then, is a loss of the cell's ability to divide an unlimited number of times. The graphical representation of the number of cells

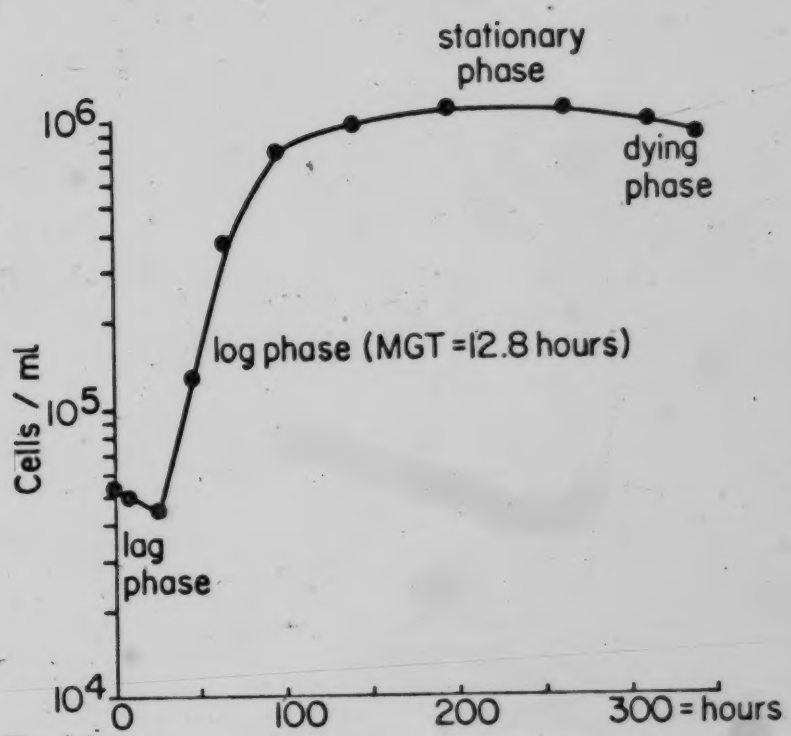


Fig.4.1 Growth of L1210 cells.

killed (whether by degeneration or reproductive death) as a function of radiation dose is called a survival curve as shown in Figure 4.2.

4.1.4 Target Theory and Survival Curves

Survival curves are obtained by placing a measured amount of an irradiated cell suspension in a test tube or petri dish containing an agar gel to immobilize the cells. After incubating the cells for a period of time, the colonies formed by viable cells may be counted. Each colony may be attributed to one viable cell. The plating efficiency of the procedure may be determined by the use of controls. This procedure for obtaining survival curves will be detailed in section 5.1.2 (B).

Survival curves may vary depending on growth conditions before and after irradiation, and on the cell line used. Most in vitro mammalian cell survival curves, however, have a basic sigmoidal shape (Elkind and Whitmore, 1967). The survival curve in Figure 4.2 shows that the relationship between cell survival and radiation dose is exponential over a certain dose range. The shoulder of the curve at low doses indicates an accumulation of sublethal damage which may be repaired by cellular mechanisms.

The target theory (Crowther, 1924; Lea, 1946) attempts to correlate the exponential dose-survival curve with the physical mechanisms of cellular radiation absorption. The site at which a primary ionization occurs in a cell is the same site at which a lesion occurs which causes inactivation or killing of the cell. The target theory predicts that the decrease in a measured function caused by radiation will be an exponential function of dose.

The basic assumptions of the target theory are that certain

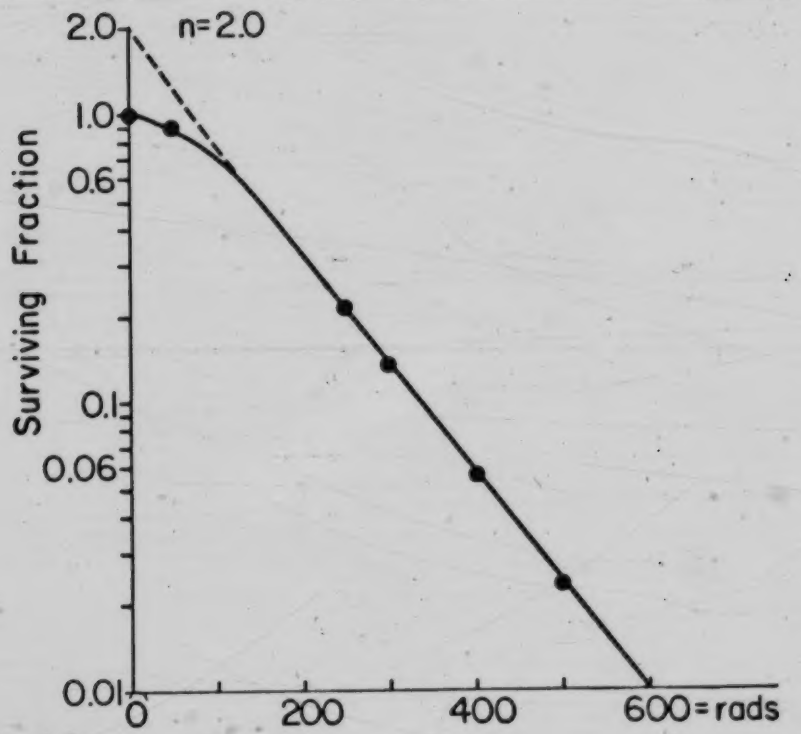


Fig.4.2 Survival of L1210 cells irradiated with γ rays.

critical sites or targets within a cell must be "hit" for the cell to be killed or inactivated. The radiation injury measured is due to one or more events within the target. These events are chemical reactions which lead to expression of a biological response and depend solely on the physical nature of the radiation. In the absence of repair mechanisms, this process is independent of the dose rate.

The target theory has many limitations. It is not applicable to the occurrence of indirect action and does not take into account the existence of cellular repair processes or the growth parameters of cells. Another limitation of the target theory is the inability to determine the target molecules.

Some target molecules have been considered, such as DNA or other nucleic acids (nucleus), cellular membranes, and mitochondria. Radiation may also interfere with cellular processes such as DNA or protein synthesis causing inactivation or cell killing in this manner.

Setlow and Pollard (1964) have derived an equation which defines the surviving ratio for the most general situation; a multi-hit, multi-target response. The surviving ratio (S) is given by:

$$S = 1 - \left[1 - \left(e^{-kD} \sum_{m=0}^{n-1} \frac{(kD)^{-m}}{m!}\right)\right]^{P}$$

where kD = average number of hits per target

n = number of hits required to kill a target

P = number of targets which must simultaneously receive n hits to be killed

4.2 Effect of Ionizing Radiation on Cells

When x-rays or γ-rays are incident on a biological material, they cause alterations in the molecules comprising the material. This initial chemical change is difficult to directly detect, and in fact, may be repaired. These chemical changes may result in a biological response, although the determination of how this occurs is not clear in all cases.

Irradiation of cellular systems can result in alterations of macromolecular synthesis and normal cell progression, cytological changes, and cell death. It has been found that mammalian cells are much more radiosensitive than even biologically sensitive molecules. Doses of 100 rads may be lethal to a mammalian cell, whereas this same dose would only cause slight damage to the macromolecule (Watanabe and Okada, 1966).

A given radiation dose may be administered either all at once, or the total dose may be split into a number of parts with refractory periods between doses. If the total irradiation time from the first to the last dose takes a matter of days or weeks, the exposure is referred to as fractionated.

If a certain amount of radiation injury must be accumulated to cause cell death, some of the initial damage will be repaired before the lethal amount of damage is produced if repair processes begin immediately after production of an injury. Thus, when the total radiation exposure time is increased, more repair of early damage will occur. This is the basis for fractionated doses. By using a fractionated dose technique and measuring cell survival after irradiation, Elkind and Sutton (1959, 1960) demonstrated that mammalian cells were able to repair sublethal radiation damage. The non-irradiation time period between doses enables the cells to repair any accumulated sublethal damage. However, this does

not imply repair of lethal damage (Elkind and Whitmore, 1967). Repair of sublethal damage coincides with restoration of the survival curve shoulder. Recovery from sublethal damage is affected by variations in the radiosensitivities of different cell stages and cell lines, duration of the non-irradiation time interval, total dose, and the total exposure time.

Cultured cells which have been exposed to post-irradiation growth conditions have shown alterations in growth and survival characteristics (Okada, 1970). The fact that cell survival can be affected by post-irradiation treatment indicates that recovery from sublethal damage and expression of potentially lethal damage are competing processes (Phillips and Tolmach, 1966).

A decrease in cell survival after post-irradiation treatment can result from suppression of repair mechanisms or the confirmation of potentially lethal damage. Chemical substances which inhibit macromolecular synthesis (i.e., puromycin) can cause a decreased cell survival (Okada, 1970).

An increase in cell survival after post-irradiation treatment can result from alterations in medium content, reduced temperature (Winans et al., 1972), or addition of chemical substances (i.e., cyclohexamide) to the cellular environment. This increase in cell survival is due to enhancement of repair processes or suppression of the expression of potentially lethal damage.

DNA is most likely the critical target in cellular systems. Radiation doses which completely inhibit cellular replication have no effect on non-dividing cells (Dertinger and Jung, 1970). As well as DNA,

the cell membrane might also be a structure that could be altered by the occurrence of one or more energy absorption events. After radiation-induced damage of cellular membranes, enzymes may be released which destroy important cellular structures by enzymatic attack (Bacq and Alexander, 1961). However, there has been no significant degradation of other cellular components found in cells even after high radiation doses in which degradation of DNA has been observed after irradiation. This degradation of DNA probably results mostly from repair of radiation damage. It has also been shown that cell membrane permeability to substances changes insignificantly after high doses of ionizing radiation (Bacq and Alexander, 1961).

Radiation also affects the normal growth processes in cells. DNA, RNA, and protein synthesis may still take place, although patterns and rates of synthesis may be altered (Elkind and Whitmore, 1967).

A decrease in the rate of DNA synthesis and prolongation of the synthetic phase has been noted after irradiation of cells. The effect of the ionizing radiation on DNA depends on dose, duration of exposure, and the stage of the cell cycle. The total amount of DNA synthesized, however, is at least as great as that in control cells (Weiss, 1971).

In mouse leukemic cells, DNA synthesis in S phase cells is depressed almost immediately upon irradiation. This depression lasts for approximately one to two hours; synthesis recurs in two to five hours after irradiation and is approximately 20% higher than in S phase control cells (Whitmore et al., 1961). This increase in DNA synthesis has been attributed to repair processes.

Repair of damaged DNA after irradiation with moderate doses (repair

synthesis) occurs not only in S phase, but in all phases of the cell cycle. This is referred to as "unscheduled DNA synthesis," and is dose-dependent. Repair synthesis occurs in both template and replicating DNA strands; subsequently, the repaired DNA undergoes replication in S phase (Brent and Wheatley, 1971). This indicates that functional integrity has been maintained.

With irradiation of 500 rads, most cells complete the first generation after irradiation, but about 25% of those cells are arrested permanently in mitosis after this generation. DNA synthesis in those cells which do not divide further was only 30% of the normal amount during the first generation compared with 80% in cells which divide (Hopwood and Tolmach, 1971). This deficiency in DNA synthesis is associated with reproductive cell death and may be due to DNA structural damage (Hopwood and Tolmach, 1971).

Total RNA synthesis (mRNA, tRNA, and rRNA) is less radiosensitive than DNA synthesis (Elkind and Whitmore, 1967). RNA synthesis in mouse leukemic cells in vitro continues at a normal rate for one generation after irradiation, even with doses up to 2000 rads (Whitmore et al., 1961). RNA synthesis is greatly reduced for a long period of time in subsequent generations. For other cell lines, this may not be the case, and in fact, RNA synthesis may be unaffected by the radiation or even slightly stimulated.

There are two sites in the cell cycle where radiation can alter the normal cell progression. These are S phase and late G_2 phase. In late G_2 phase, a temporary block occurs which appears to be caused by insufficient synthesis of specific proteins needed for mitosis (Doida and Okada, 1969). This has been substantiated by experiments using the

antibiotic puromycin which inhibits protein synthesis by releasing polypeptide chains before their synthesis is completed. The presence of puromycin blocks protein synthesis at the same point as the radiation-induced block at G_2 (Doida and Okada, 1969) and also prevents recovery from the block. This G_2 block is a factor in causing mitotic delay but should not affect the reproductive death of cells.

After irradiation, the normal presence of mitotic cells and cell division is suppressed for a period of time referred to as division delay or mitotic delay. The length of this delay is dependent on radiation dose, type of radiation, cell type, and the physiological state of the cells at the time of irradiation (Elkind and Whitmore, 1967). At the end of the mitotic delay, the cells which have not lost their reproductive ability begin to divide an indefinite number of times. These cells give rise to colonies when grown in an agar medium. Some cells may divide a limited number of times and give rise to abortive colonies (Puck and Marcus, 1956). Some radiation-damaged cells may lyse before the first mitosis. Other cells continue to synthesize cellular material, but fail to divide, and thus give rise to large multinucleate cells, or giant cells. These cells undergo mitosis with division and then immediate fusion, or fail to undergo mitosis at all (Okada, 1970). These mitotic processes are not mutually exclusive, but may occur together. The mitotic delay can be determined for asynchronous cell cultures by a decrease in the relative number of cells undergoing mitosis (decline in the mitotic index) or by a cessation of multiplication after irradiation. Cells are most sensitive to radiation-induced mitotic delay in S, G_2, and G_1 phases in order of decreasing sensitivity (Sinclair, 1968).

The survival response of a cell after irradiation depends on the position of the cell in the growth cycle at the time of irradiation. Cells are most sensitive to radiation-induced reproductive death in M and G_1 phases, and most resistant in S phase. During the S phase, repair of DNA can occur, as evidenced by an increase in DNA synthesis. There is a radioresistant peak in late S phase which indicates an association between DNA synthesis and the survival peak (Sinclair, 1969), albeit indirect. When synthesis of protein, DNA, and RNA is prevented from occurring early in the cell cycle, i.e., in G_1 phase, the survival peak is depressed in late S phase, indicating that all macromolecular synthesis must proceed normally (Sinclair, 1968). It has also been determined that when all macromolecular synthesis is inhibited, changes in cell survival can occur. Thus, there must be some other cell cycle-dependent substance or factor which influences cell survival (Sinclair, 1969).

Cells may metabolize through several cycles before dying and thus cellular damage may be amplified. If damage is done to the DNA template, this may result in faulty macromolecular synthesis (Hopwood and Tolmach, 1971) or structural damage to cellular or subcellular membranes (Okada, 1970).

Ionizing radiation can produce structural changes in chromosomes at any stage of the cell cycle (Lea, 1946; Elkind and Whitmore, 1967). Since the chromosomes are visible only during mitosis, chromosomal aberrations must be noted during this stage. This is most easily done during metaphase or anaphase when the chromosomes are short, compact, discrete structures.

Chromosomes are composed of two chromatids. At the end of the presynthesis phase or G_1, the chromatids separate, and then replicate

during S phase. During mitosis, each of the two chromatids, together with its complementary replicated chromatid, becomes the chromosome in each of the two daughter cells.

Aberrations in chromosomes are classified according to the portions of the chromosome which are initially involved in an alteration due to ionizing radiation. A chromatid aberration may occur after replication has taken place, in G_2 or S phase, when the chromatids are separated. If both chromatids in a chromosome are affected at identical loci due to a single break, then this is referred to as a chromosome aberration. This type of aberration occurs before replication during G_1 or S phase, before the two chromatids have separated.

The frequency of occurrence of both types of aberrations depends on the phase of the cell cycle, the LET of the radiation, total dose and dose rate, the chromosome number of the karyotype, and the physical and chemical environment of the cells during irradiation. There also appears to be a correlation between chromosomal aberration and cell survival (Dewey et al., 1972).

4.3 Effects of Microwave Radiation on Cells

Many studies have been done on the effect of microwave radiation on cell cultures in vitro. The experiments have been divided into two categories: those studying hyperthermic effects and those investigating nonthermal effects. In most cases, especially with higher intensity microwaves, it is difficult to determine the existence of any nonthermal effects since they are easily masked by hyperthermia and its resultant biological consequences.

Microwave effects on cells have been reported by researchers to be both stimulating and inhibiting. The wide variety of results stems from the use of a large range of microwave frequencies, intensities, and exposures, and from the diversity of cell cultures used in the experiments. Some of the observed changes which have occurred with low incident power densities include: lymphoblastoid transformation (Stodolnik-Baranska, 1967, 1974); changes in ion flow across a membrane (Barnes and Hu, 1977); increased membrane permeability (Baranski et al., 1974); formation of large malformed cells (Valtonen, 1966); inhibition of cell growth and chromosomal damage (Heller, 1970); orientation of blood cells, unicellular organisms, and colloidal particles along the lines of force of an electric field (Herrick, 1958); and anatomical and structural aberrations in developing embryos (Van Ummersen, 1961).

Hyperthermic cellular effects produced by high intensity microwaves have been widely studied for the last few decades (for review, see Thrall et al., 1976, or Har-Kedar and Bleehan, 1976). It has been found that cell survival decreases with elevated temperature and that the rate of cell death depends on the cell type, cell cycle, and phase of growth. Several targets have been suggested as a cause of cell lethality by hyperthermia: DNA (Westra and Dewey, 1971); RNA (Dickson and Shah, 1972); proteins (Westra and Dewey, 1971); membranes (Mondovi et al., 1969); and lysosomes (Mondovi et al., 1969; Overgaard and Overgaard, 1972). The effectiveness of repair mechanisms after hyperthermic exposure can also affect the degree of lethality.

Tumor cells in vivo have been found to be more sensitive to hyperthermia than cells in normal tissues. This may be due to many factors: poor vascularization in areas of tumor cell; intrinsic differences in

cell susceptibility; a specific response of cells in S phase; or to the absence of protection from hypoxic conditions (Giovanella et al., 1970; Cavaliere et al., 1967; Overgaard and Overgaard, 1972; Hahn, 1974; Westra and Dewey, 1971).

Gross anatomical and structural aberrations have been found in developing embryos as a result of low intensity microwave radiation (Van Ummersen, 1961). This and other experiments have indicated that undifferentiated cells are potentially more sensitive to microwave and radiofrequency radiation than differentiated cells.

Cellular membranes have been suggested as a site of low intensity microwave interaction with cells (Baranski et al., 1974; Barnes and Hu, 1977). Biological membranes are sheet-like structures that are composed of proteins and lipids held together by noncovalent interactions. Membranes separate cells from their environment and create organelles within a cell (i.e., mitochondria, nuclei, lysosomes, and chloroplasts). They are highly selective permeability barriers which regulate the ionic and molecular composition of the cell or organelle. Membranes control the flow of information between cells and their environment and some membranes also play a role in energy conversion processes.

Barnes and Hu (1977) have shown that shifts in the ion concentration across a cell membrane can occur as a result of microwave and radiofrequency fields, which in turn can affect the membrane permeability. Any impairment of the membrane permeability can cause a shift of molecules from one compartment to another resulting in disturbance of metabolic processes. This can lead to cell death.

If an electric field of 1 kV/cm in free space is incident on a biological material of high water content with membranes at right angles

to the field, the field strength in the membrane is 2.26×10^3 V/cm. For a 200 Å thick membrane, the voltage across the membrane is 4.5 mV (Barnes and Hu, 1977). This leads to ion currents of approximately 10^8 ions per second across a surface area of 10 μm on the side (area = 10^{-6} cm^2). This ion current is large enough so that a significant number of charged ions or molecules may be moved across a membrane in a short period of time. This could result in biological alterations within the cell (Barnes and Hu, 1977) or metabolic disturbances.

At a power density of 10 mW/cm^2, the field strength in free space is 1.94 V/cm and in a material of high water content containing the biological membranes, the field strength is 4.4 V/cm. The corresponding voltage drop across the membrane is 9 μV (Barnes and Hu, 1977). This corresponds to an ion flow of about 4×10^8 ions/cm^2/sec or to 400 ions per second for a cell with a 10^{-6} cm^2 surface area. This ion current is small enough that its biological significance is doubtful (Barnes and Hu, 1977).

Irradiation of granulocytes and erythrocytes in vitro resulted in marked injury of cell membranes (Baranski et al., 1974). Exposure to 10 cm microwaves at a power density of 1 mW/cm^2 (too low to cause heating) resulted in increased potassium, hemoglobin, and phosphatase diffusion out of the cells. This was followed by a decrease in the osmotic resistance of the cells. At higher power densities, there was an increased cell membrane permeability for hemoglobin (Baranski et al., 1974). Injury to cell membrane function appears to be both time and dose dependent.

The interaction of microwaves on excitable cells is connected with alterations in the permeability of cell membranes. This is brought about

by changes in the transport of ions which affect the membrane potential (Gordon et al., 1973). The mechanism by which these changes occur is not known.

Microwave radiation is the only physical agent so far that has been known to cause lymphoblastoid transformation of in vitro lymphocytes (Stodolnik-Baranska, 1967). Changes from lymphocytes to lymphoblasts, which are lymphocyte precursors, were accompanied by aberrations in the cell nucleus and in the mitotic activity of the cell (Czerski, 1974). The most common nuclear aberrations were aneuploidy, hyperploidy up to 3-4 n, chromatid breaks, and dicentric and acentric chromosomes (Stodolnik-Baranska, 1974). These aberrations occurred with incident microwave radiation with a 10 centimeter wavelength and incident power density of 7-14 mW/cm^2 for up to three exposures of four hours each. Similar results occurred in cultures irradiated at a power density of 20 mW/cm^2. There was no rise in temperature of the cell cultures above 38° C, thus the effect can be attributed to the effect of microwave irradiation and not to thermal effects (Stodolnik-Baranska, 1967). At power densities above 20 mW/cm^2, only heating and cell death occurred (Baranski and Czerski, 1976). Degeneration and cell death usually occurred with temperature increases of approximately 3° C.

Heller (1970) has found that human lymphocytes subjected to pulsed radiofrequency waves (100 pulses per second, 10 μsecond per pulse) at 21 MHz with 500 volts peak-to-peak per centimeter for 30 minutes caused an increase in the incidence of chromosome abnormalities as compared to a control. The temperature during the irradiation was maintained at 27° C so thermal effects could be ruled out. The chromosomal damage included single chromatid breaks and dicentric and acentric chromosomes.

There was also some evidence of non-dividing nuclei.

Pearl chain formation, which was discussed previously does not appear to be significant for in vivo biological systems. Unicellular organisms, such as amebae, euglena, and paramecia, exhibit this phenomenon in low intensity radiofrequency and microwave fields. Cells in suspension, such as leukocytes and erythrocytes also align themselves with the lines of force of an applied electric field. Schwan (1958) has postulated that long chain molecules with dipole moments, such as biological membranes or macromolecules, may also be aligned along electric field lines. However, the existence of this effect and its biological consequences has not been shown.

The evidence for the existence of nonthermal cellular effects is growing and with it the conflicting reports on the mechanism of low intensity microwave effects. It still remains to be proven that nonthermal cellular effects can be caused by low intensity microwaves and to determine the mode of interaction between the electromagnetic field and the cell.

4.4 Combined Effects of Microwaves and Ionizing Radiation

Hyperthermia has long been known to enhance the sensitivity of cells in vivo or in vitro to ionizing radiation. This is applicable also to tissue heating caused by radiofrequency or microwave radiation used in combination with x- or γ-irradiation. This synergistic effect may occur because proteins and enzymes are very sensitive to any temperature increases. Any disruption in cellular function may lead to an increase in sublethal or lethal cellular damage. After exposure to ionizing

radiation, sublethal damage may become lethal if repair mechanisms are unable to function.

During experiments with combinations of hyperthermia and low dose rate ionizing radiation it was found that the thermal effect on cell death varied inversely with the dose rate (Ben Hur et al., 1972). The greatest effect was found when the cells were subjected to an elevated temperature of 41° C for six hours and then exposed to ionizing radiation. This pre-treatment with hyperthermia impaired the cellular system responsible for repair of sublethal damage (Harisiadis et al., 1978). Maximum cell killing by ionizing radiation was observed since the repair of sublethal damage was greatly reduced or did not occur. When low dose rates were used, cell killing was reduced due to the repair of sublethal damage during the longer exposure time. If cell cultures were exposed to low dose rate ionizing radiation before hyperthermic treatment, the repair mechanisms functioned normally during irradiation and were impaired only during the hyperthermic treatment. This did not lead to an increase in cell killing. Simultaneous hyperthermia and low dose rate irradiation led to an intermediate effect since the impairment of repair mechanisms evolves gradually and some repair may occur in the early stages of irradiation (Harisiadis et al., 1978). Heating the cell cultures to 41° C did not kill a noticeable number of cells.

Mammalian cells are most sensitive to hyperthermia during the S phase of the replication cycle, that phase where cells are most radio-resistant to ionizing radiation. In addition, tumor cells are more sensitized to ionizing radiation by hyperthermia than are cells in normal tissues (Crile, 1963; Hofer et al., 1977).

Microwaves have also been shown to affect the sensitivity of cells, especially undifferentiated cells, to ionizing radiation. Exposure to 100 mW/cm^2 2800 MHz pulsed microwaves decreased the cell mortality from subsequent x-ray exposure (Michaelson et al., 1962, 1963). This also occurred for microwaves with incident power density of 165 mW/cm^2. Simultaneous x-ray and microwave exposure, or microwave exposure following x-irradiation, which may reveal the residual radiation damage, caused adverse effects. Howland and Michaelson (1964) have postulated that microwave exposure results in changes to the compensatory and homeokinetic mechanisms of the organisms.

CHAPTER V

EXPERIMENTS

5.1 Methods and Materials

5.1.1 Routine Procedures

The cells used in this study were lymphocytic mouse leukemia L1210 cells (Cass and Au-Yeung, 1976) obtained from Dr. A. R. P. Paterson of the McEachern Cancer Research Unit at the University of Alberta. Reserve L1210 cell cultures are kept frozen in ampuls in liquid nitrogen (-196° C) at the McEachern Laboratory where a new culture is thawed every several months to minimize alterations in the characteristics of cells carried in vitro. This maintains the purity of the cell line (Willmer, 1965). Stock cultures of L1210 cells were maintained in vitro by redilution into fresh culture medium every two to three days (Moore, Sandberg and Ulrich, 1966). The culture medium used to maintain the stock cultures consisted of 90% Fischer's medium and 10% horse serum (Grand Island Biological Company, Grand Island, New York) (Fischer and Sartorelli, 1964) buffered with sodium bicarbonate ($NaHCO_3$). The Fischer's medium was made from liquid sterile 10X concentrated Fischer's medium added to nine parts distilled water and buffered with 1.125 grams sodium bicarbonate per liter of medium. The culture medium was sterilized by filtering with a 22 μ Millipore filter (Millipore Corporation, Mississauga, Ontario). The composition of Fischer's medium and horse serum is shown in Table IX. Antibiotics were not used in the medium for maintaining stock cultures

TABLE IX

CULTURE MEDIUM COMPOSITION

1. 90% Fischer's Medium (from Gibco Catalogue) with 1.125 g/L $NaHCO_3$

	mg/L		mg/L
$CaCl_2 \cdot H_2O$	91	methionine	100
KCl	400	phenylalanine	60
$MgCl_2 \cdot 6H_2O$	100	serine	15
NaCl	8000	threonine	30
$NaH_2PO_4 \cdot H_2O$	69	tryptophan	10
$Na_2HPO_4 \cdot 7H_2O$	113	tyrosine	20
glucose	1000	valine	70
phenol red	5	biotin	0.01
arginine	15	Ca pantothenate	0.5
asparagine	10	choline chloride	1.5
cystine	20	folic acid	10
glutamine	204	i-inositol	1.5
histidine·HCl	66	nicotinamide	0.5
isoleucine	75	pyridoxal HCl	0.5
leucine	30	riboflavin	0.5
lysine HCl	50	thiamine	1.0

2. 10% Horse Serum (from Altman and Dittmer, 1961)

Lipids (cholesterol)
Carbohydrates (glucose)
Proteins (albumin, γ-globulin)
- lipoprotein
- glycoprotein
- enzymes (cholinesterase)
- hormones (thyroid, gonadotrophic, lactogenic hormones)

Non-protein nitrogenous substances
- amino acids (cystine, tryptophan, cysteine)
- bilirubin
- urea

Electrolytes (Ca, Cl, Cu, K, Na, Mg, S, Si, phosphate)
Vitamins (A, C, folic acid, pantothenic acid, choline)

3. Antibiotics

Penicillin	10,000 units/ml
Streptomycin	10,000 µg/ml
Gentamicin	10 mg/ml

in order to prevent the development of drug-resistant microorganisms, and to discourage the use of careless sterile technique (Raul, 1975). If contamination of the cell cultures occurred, it was noticeable within a few days and the stock culture was discarded and fresh cells were obtained. Periodic checks for contamination were made by streaking a blood agar plate with a sample of the cell culture, incubating, and checking for bacterial contamination.

To plot a growth curve for L1210 cells, a sample from a stock culture was placed in a bottle containing culture medium to a concentration of approximately 5×10^4 cells/ml. The medium containing the cells was placed in an incubator at the desired temperature (generally 37° C), and the cells were allowed to grow until they reached saturation. One milliliter samples were removed under sterile conditions at various times during the life of the cells and diluted into an isotonic saline solution (Isoton, Coulter Electronics of Canada Limited, Mississauga, Ontario) to be counted. Cell numbers were counted using the Coulter Counter Model F_N (Coulter Electronics of Canada Limited). A growth curve was then obtained for each cell culture by plotting total cell number as a function of incubation time. This normal curve for L1210 cells was repeated during every experiment as a control.

Two stock cultures of L1210 cells were maintained during the course of the experiments. Cell samples were taken from the stock cultures for use in experiments. For the purposes of short-term experiments, the cells were grown in the culture medium with 1% of the total volume consisting of the antibiotic penicillin (10,000 units/ml) - streptomycin (10,000 µg/ml) (Grand Island Biological Company) to deter bacterial contamination. For long-term experiments 1% gentamicin reagent

solution (10 mg/ml) (Schering Corporation, Kenilworth, N.J.) was used as as antibiotic.

The conditions for optimum cell growth were first determined for L1210 cells by varying the environmental conditions. It was established that L1210 cells grew optimally at an incubation temperature of 37 ± 1° C and with an atmosphere consisting of 5% CO_2 and 95% air with 90% relative humidity. Cells suspended in medium were contained in 60 ml bottles with loose fitting caps open to the atmosphere in the incubator. Every second day, the cell culture was counted and diluted to 5×10^4 cells/ml. The lag phase was minimized by maintaining the concentration of the stock culture between 5×10^4 cells/ml and 5×10^5 cells/ml so that the cells were growing logarithmically at the time of dilution into fresh medium. The mean generation time (MGT) was used as a measure of growth. The MGT is a measure of the number of doublings that occur in a cell population during a time interval $\Delta t = t_2 - t_1$.

$$MGT = \frac{t_2 - t_1}{\log_2 N_2 - \log_2 N_1}$$

where N_1 = cell population at time t_1

N_2 = cell population at time t_2

(See Appendix I for calculation of MGT.)

All glassware used in these experiments was sterilized by dry heat at 250° C, 20 pounds pressure, for 30 minutes. Pipets, culture tubes, and petri dishes were sterile and disposable.

5.1.2 Irradiation of Cell Suspensions with γ-Rays

(A) Growth curves

The following procedure was used to obtain growth curves for cells exposed to γ-rays. A volume of 20-30 ml of cells at a total concentration of approximately 5 x 10^5 cells/ml contained in a petri dish was placed on a 0.5 cm thick plastic sheet positioned on top of the outermost collimator vanes of an eight kilocurie Cobalt-60 teletherapy unit (Theratron 80) (average γ energy of 1.25 MeV). The cell suspension was irradiated from below through the sheet of plastic to ensure full build-up. The dose rate at the point of irradiation was determined by the use of an Ionex dose/dose rate meter with a Baldwin-Farmer 0.6 cc ion chamber. The activity of the cobalt source decays at approximately 1% per month and this was taken into account in subsequent experiments. Periodically the dose rate was reconfirmed by the same method. The cells were given doses between 250 and 4000 rads. The dose delivered to the cells was determined by dividing the desired dose by the dose rate and obtaining the time of exposure needed for the cells to receive a given dose. A correction factor of 0.01 second was added to the time to correct for the shutter speed.

Thermoluminescence dosimetry (TLD) was used to reconfirm the dose rate at selected points of irradiation. Samples of lithium fluoride (^{7}LiF) powder were irradiated by the Cobalt-60 source from below, through the 0.5 cm sheet of plastic, and given doses corresponding to 100 and 200 rads as previously determined. The total light output of the samples was read using a Harshaw 2000 A thermoluminescent detector with a Harshaw 2000 B automatic integrating picoammeter. The total light output, which

is proportional to the radiation dose received, was compared to the light output of a sample that was irradiated to a known dose of 100 rads. The values were within 10% of each other.

After the cell suspension had received the required dose, 2 ml of the suspension was removed and resuspended in 18 ml of fresh medium to obtain a cell count of approximately 5×10^4 cells/ml. The cells were counted immediately after irradiation, placed in an incubator at the desired temperature, and subsequently counted in intervals of approximately 24 hours for a week or until the cultures had reached stationary phase. The total cell number was graphed as a function of incubation time and thus the growth curve was a direct record of the effect of γ-rays on L1210 mouse lymphoma cells. The growth curve for an experiment enables both cell progression through replicative cycles and cell death to be noted. The fraction of irradiated cells which are able to grow and divide can be compared with the control growth curve to determine the magnitude of the radiation (γ-ray or microwave) effect.

There were two controls for each experiment, C_1, under sterile laboratory conditions, and C_2, under actual experimental conditions. Both control samples were also grown with 1% penicillin-streptomycin added to the medium.

(B) Survival curves

Mouse leukemia L1210 cells will form colonies of cells in a soft agar medium (Himmelfarb, Thayer, and Martin, 1967). This procedure can be used to determine cell viability since one viable cell will form one colony. For L1210 cells which are irradiated with γ-rays, the number of colonies formed can be used to describe the cell killing or loss of cell

viability. This was done by comparison with a control culture which had not been exposed to γ-rays. The number of colonies formed in agar for a given dose was expressed as a percentage of the control values. The graphical representation of the logarithm of the surviving fraction of cells (which is directly proportional to the number of colonies formed) as a function of dose is referred to as a survival curve (Elkind and Whitmore, 1967).

The procedure for formation of L1210 colonies in soft agar has been modified from a technique developed by researchers at the McEachern Laboratory (Cass and Au-Yeung, 1976). It was originally developed for determination of cell viability after drug exposure, but may also be used to study viability after exposure to physical agents, such as non-ionizing or ionizing radiation.

The procedure for obtaining survival curves for L1210 cells after irradiation with γ-rays was as follows. Log phase L1210 cells at a concentration of 1×10^5 cells/ml were irradiated by the same method as that used to obtain growth curves in section 5.1.2 (A). After a given dose had been delivered to the cells, a one milliliter sample of cells was removed and diluted into cloning medium. The cloning medium consisted of 20% horse serum, 59.5% Fischer's medium, 0.5% gentamycin, and 20% conditioned medium. Conditioned medium has growth-enhancing characteristics and was obtained from medium in which cells have been growing for 48 hours. This was filtered to remove cells in suspension by pouring the medium into a 22 μ Millipore filter on the day of the experiment. Generally, the cell sample was given a range of doses varying from 50 to 8000 rads.

A succession of dilutions into cloning medium was carried out for each dose until the desired concentration was reached. This enabled plating a specific number of cells per tube. The final concentration was predetermined to obtain a countable number of colonies (50-100) per tube. A 2.5 ml sample of cells at the final concentration was then added to 2.5 ml of 0.26% Noble Agar (Difco Laboratories, Detroit, Michigan) and 2X Fischer's Medium with 1% gentamycin, and placed in a sterile 16 x 125 mm test tube with cap. This resulted in many test tubes corresponding to various doses (generally 4-8 tubes per dose) containing 5 ml of cells in cloning medium and 0.13% agar with the desired theoretical number of cells per tube. The test tubes were placed on ice for 30-60 seconds to solidify the agar and then incubated at 37° C with 5% CO_2 and humid air for 12-14 days. To count the number of colonies, the soft agar was poured into a small petri dish and scanned under a low power magnifying glass in a Darkfield Quebec Colony counter (American Optical Corporation, Buffalo, New York). The plating efficiency for this cell line is given by the number of colonies formed in agar expressed as a percentage of the theoretical number of cells per tube. The survival curves obtained in this manner are then used to determine the magnitude of the effect of the radiation.

5.1.3 Exposure of Cell Suspensions to 2450 MHz Microwaves

(A) Microwave equipment and dosimetry

The microwave apparatus used in these experiments was obtained from Dr. Wayne Tinga of the Electrical Engineering Department at the University of Alberta. It consisted of an S712 signal source

(PRD Electronics, Inc.), two coaxial directional couplers (Waveline, Inc.) a Micromega isolator, coaxial cable (RG-8/U, Canada Wire and Cable Company, and RG-214/U, Hewlett Packard), and a 2450 MHz tunable copper waveguide. The system and details of the rectangular waveguide are diagrammed in Figure 5.1. The oscillator was adjusted to generate a 2450 MHz continuous wave with 100 mW power density. A Hewlett Packard 435A Power Meter, connected by a power sensor cable to an HP 8481 A Power Sensor, was used to measure the microwave power.

A directional coupler is a device consisting of two transmission lines coupled together in such a way that a wave travelling in one line in one direction excites a wave in the other line. When separated by an isolator, the two directional couplers in the circuit are positioned so that forward and reflected power can be measured separately. The directional couplers used in this system sampled 1% of the main line power. These are shown in Figure 5.1.

The incident power to the sample in the waveguide is determined by the difference between the forward and reflected power. When a cell suspension is placed in the waveguide, the absorbed power in the cell suspension depends on the volume and on the position of the petri dish in the waveguide. For each experiment, the petri dish containing 20 ml of cells in suspension was placed in the center of the waveguide. The tuner was adjusted so that there was minimum reflected power. As one milliliter samples were removed from the petri dish, the total absorbed power in the sample decreased. Forward power ranged from 70 mW to 78 mW as sample volume increased from 10 to 20 ml. The reflected power ranged from a maximum of 10 mW with 10 ml of cell suspension to a minimum of

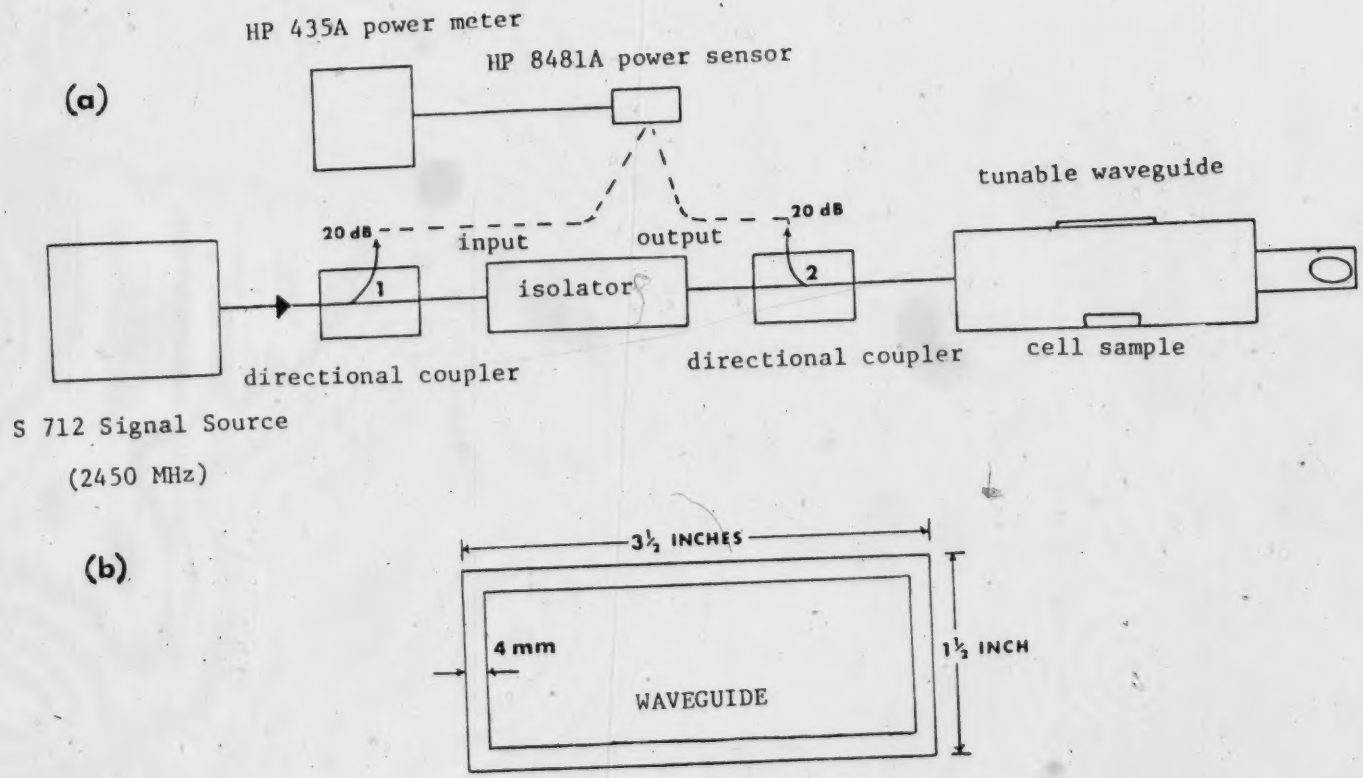


Fig. 5.1 Microwave apparatus (a) Schematic diagram of the system. Points 1 and 2 indicate the directional couplers where the forward and reflected power are measured. (b) Waveguide dimensions are indicated.

5 mW with 20 ml of cell suspension. The difference between forward and reflected power in the sample thus ranged from a maximum of 73 mW with 20 ml of cell suspension to a minimum of 60 mW with 10 ml of cell suspension. When the sample was removed from the waveguide, the reflected power was within 15% of the forward power, allowing for power losses (i.e., waveguide, coaxial cable, directional coupler).

Provided that a biological system absorbs sufficient energy that its overall temperature increases, microwave dosimetry is not a problem. In this case either a calorimeter or bolometer can be used, and the absorbed energy can be directly calculated. However, for measuring low intensity microwave absorption where there is no measurable temperature increase, a semi-empirical approach must be taken.

For these experiments, the absorbed power density (P_i in mW/cm^2) in the sample can be approximated by the equation (Courtney et al., 1975; Chen and Lin, 1978):

$$P_i = \frac{(W_i - W_f)\, e^{-2\beta z}}{A}$$

where W_i = forward power

W_f = reflected power

A = 5.08 cm^2 = cross-sectional area of petri dish

β = 53.43 Np/m = .5343 Np/cm = attenuation coefficient for phosphate buffered saline (Chen and Lin, 1978)

z = 2.54 cm = radius of petri dish (effective)

The specific absorption rate (SAR in W/kg or mW/g) is the rate of energy absorption per unit mass and is related to the incident power by

the relationship (Courtney et al., 1975; Chen and Lin, 1978):

$$SAR = \frac{4\beta P_i}{\rho}$$

where $\rho = 1.01\ g/cm^3$ = density of cell suspension (PBS)

The attenuation coefficient β has been determined by Chen and Lin (1978) to be $\beta = 53.43$ Np/m or 0.5343 Np/cm in phosphate buffered saline. The attenuation of the microwave radiation in the petri dish is not included since the mass of the plastic is minimal in comparison with the mass of the solution.

For these experiments, the absorbed power density in the cell suspension ranged from 0.8 mW/cm^2 to 1.0 mW/cm^2 corresponding to a specific absorption rate of 1.7 mW/g to 2.0 mW/g.

In order to determine if this power absorption caused a temperature rise in the cell suspension it was necessary to measure the temperature of the system experimentally. The temperature of the cell suspension was monitored throughout the course of the experiments. At this low power density of approximately 1 mW/cm^2 no heating was expected to occur since the cell suspension was exposed to microwaves at room temperature (22.5° C) which is a decrease of 14.5° C from the incubation temperature. As microwave energy is absorbed by the cell suspension, any increase in temperature would be compensated for by the dissipation of heat as the sample cooled down to room temperature.

(B) Experimental procedure

The waveguide used in these experiments was modified to enable placement of a petri dish in the center of the waveguide. A circular

hole, approximately 7 cm in diameter, was cut in the top of the waveguide, and a snug fitting metal cap was made for the opening so that the inner surface of the waveguide did not have any irregularities. A 60 x 15 mm petri dish, containing approximately 20 ml of cells, was placed in the center of the waveguide and exposed to microwaves for varying periods of time up to one hour. Cell samples were removed from the petri dish at given time intervals and diluted into fresh medium. These cell cultures were incubated at 37° C. Cell counts were made periodically to obtain growth curves for cells exposed to microwaves. Control growth curves were obtained by setting an identical petri dish at room temperature and removing small samples into fresh medium at the same time intervals as for the cells exposed to microwaves. For both the control and microwave exposure, the temperature and pH were recorded at the time cell samples were removed from the petri dishes for redilution. The temperature was monitored using a Markson digital thermometer Model 5650 (Markson Science Inc., Del Mar, California). At various times during the exposure of the cells to microwaves, the field was turned off to enable the placement of the temperature probe in the waveguide and to remove cell samples. The pH was monitored at the same time using a Markson Model 74 Mini pH-meter.

Cell samples were also placed in a water bath at 40° C for periods of time up to one hour. Samples were removed from the water bath periodically and rediluted into fresh medium to obtain growth curves. This was done to determine whether slight heating would affect cellular growth, since the possibility exists for cellular microwave heating even though the microwave power density was low.

5.1.4 Combined Microwave and γ-Ray Irradiation

Several experiments were performed to determine whether microwaves would have a noticeable effect on cellular systems before or after γ-irradiation. The procedure used here was to first expose a sample of cells to a given dose of Cobalt-60 γ-rays and then place the sample in the waveguide for a predetermined time interval. After both γ-ray and microwave irradiation, a small sample of cells was diluted into fresh medium and incubated. Another sample of cells was first placed in the waveguide for the same length of time as in the previous case, exposed to the same dose of γ-rays as previously, and then a small sample was diluted into fresh medium and incubated. At the same time, a sample of cells exposed to the given dose of γ-rays alone, and a control sample of cells which had not been exposed to any radiation, were resuspended in fresh medium and incubated. Growth curves were then obtained for all these cases.

Experiments were carried out to determine the effect of simultaneous exposure to γ-rays and 2450 MHz microwaves. The microwave apparatus was set up on an adjustable support. The equipment used for simultaneous microwave and γ-ray exposure is shown in Figure 5.2. The waveguide was centered directly under the collimator vanes. The distance from the source to the top of the waveguide was measured as 69.5 cm. The source to sample distance (SSD) was 71.5 cm. The direction of propagation of γ-rays incident on the petri dish containing the cell suspension was perpendicular to the direction of propagation of the microwaves. L1210 cells were poured into a 60 x 15 mm petri dish which was placed in the waveguide. The microwave power supply

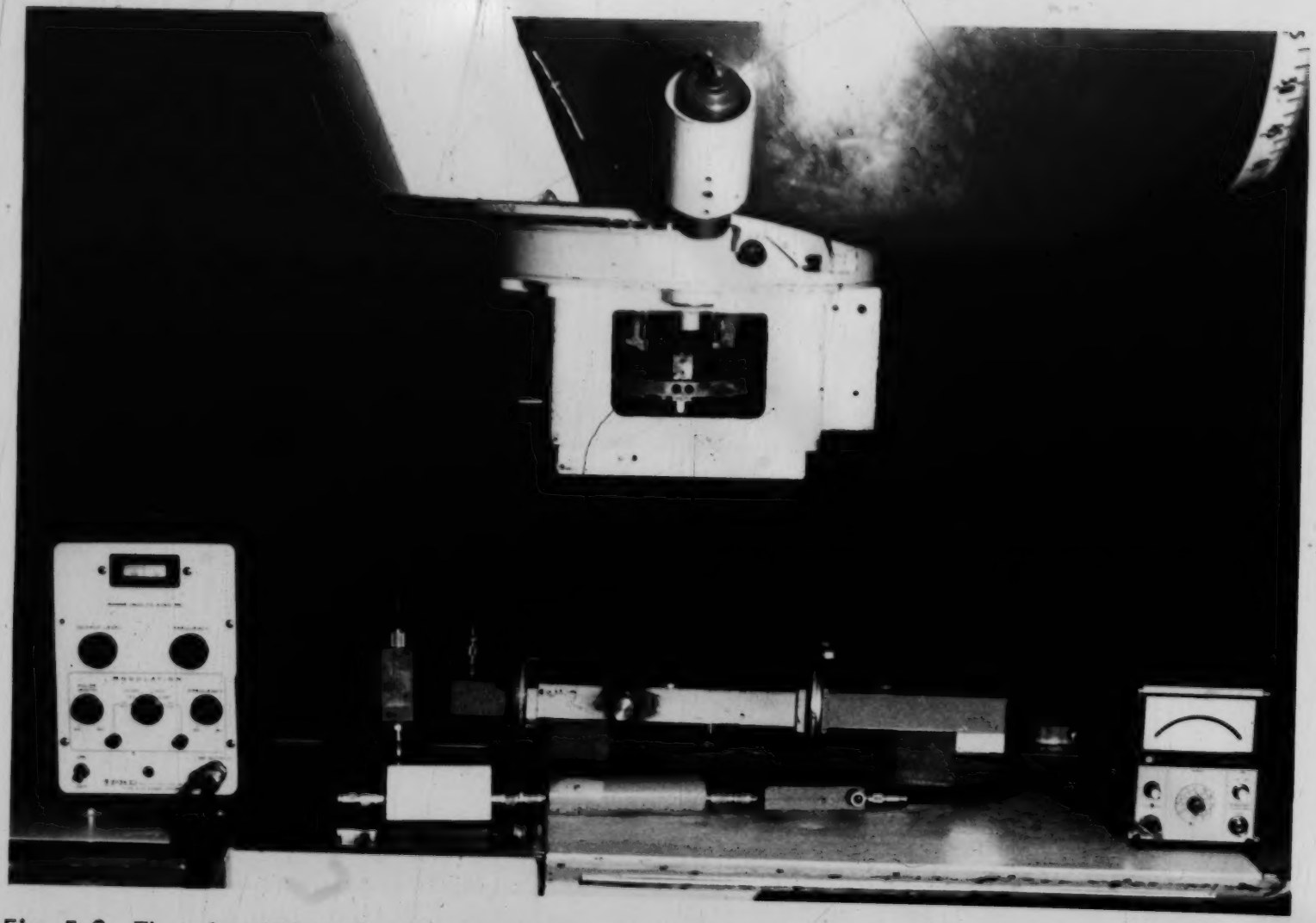


Fig. 5.2 Theratron 80 and microwave apparatus

was turned on and the cells were given various doses of γ-rays between 50 and 2000 rads. After each γ-ray dose, the microwave power supply was turned off to enable a sample of cells to be removed and suspended in culture medium. The power supply was then turned on again for the next γ-ray dose. A control experiment was done using the identical physical set-up, except the cells were not exposed to microwaves during the γ-ray irradiation even though the petri dish was placed in the waveguide. Two different techniques were used to determine the combined effects of microwaves and γ-rays by determining the fraction of a cell population which retained its viability after irradiation. The procedure used to obtain both growth curves and survival curves is the same as that used in section 5.1.2 for γ-irradiation.

The dose rate for γ-rays for this physical set-up was determined by the use of two different methods of dosimetry, the Baldwin-Farmer ion chamber read with the Ionex dose-dose rate meter and thermoluminescence dosimetry (^{7}LiF).

5.2 Results

5.2.1 Growth Curves Under Varying Experimental Conditions

L1210 cells grow optimally at an incubation temperature of 37° C in a humid (90%) atmosphere consisting of 5% CO_2 and 95% air. The growth curves in Figure 5.3 represent the normal growth of cell cultures incubated at 37° C. Throughout the course of these experiments, control growth curves were obtained periodically to determine if growth characteristics in the cell line had been altered. Control growth curves for L1210 cells over the two years of experimentation did not differ significantly. In some cases, the lag phase was more pronounced (see Figure 4.1) and cells did not grow for a period of 24 hours or actually decreased in cell number. This occurred when the cells had been allowed to grow to a high concentration in suspension, of the order of 1×10^6 cells/ml, before redilution into fresh culture medium. The cells then took longer to adapt to their environment. The MGT of the control cell cultures was determined to range from 12-18 hours under experimental conditions. Many factors contribute to this variation: inoculation cell number (generally 0.5×10^5 to 1.0×10^5 cells/ml); concentration of the cell culture from which dilution occurred; stability of the environmental growth conditions; and the length of time that the culture is removed from the incubator for cell counts to be determined. Total cell concentration for control cultures ranged from 1.2×10^6 to 1.7×10^6 cells/ml. Possible reasons for this variation include differences in the nutrient composition of the culture medium, horse serum from different batches, variations in environmental conditions, and the adaptability of L1210 cells to a new environment.

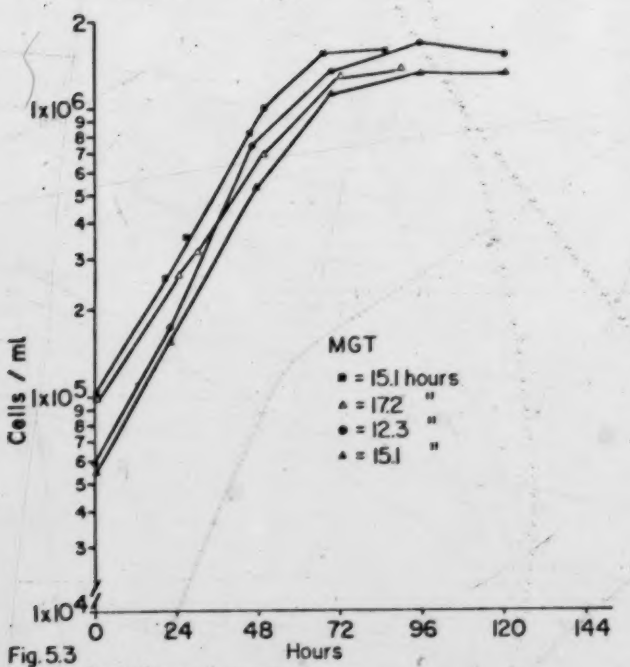


Fig. 5.3 Control growth curves.

Cell cultures were irradiated with varying doses of γ-rays under normal and reduced oxygen concentrations, and then incubated at 37° C, 39° C, and 41° C. The growth curves are shown in Figures 5.4 through 5.7. There was no growth at an incubation temperature of 41° C.

As shown in Figure 5.6, the combination of an elevated incubation temperature and γ-irradiation under oxic conditions is lethal to L1210 cells. At an incubation temperature of 39° C, a dose as low as 250 rads is sufficient to prevent cell growth. Growth curves for cells irradiated under oxic conditions and then incubated at 37° C and 39° C are shown in Figures 5.4 and 5.6 respectively.

A series of experiments were done with cell cultures irradiated under a reduced atmospheric oxygen concentration. Nitrogen gas was slowly bubbled into the covered petri dish containing the cell suspension during the period of irradiation. The cells were then incubated at temperatures of 37° C, 39° C, and 41° C in successive experiments. The growth curves for cells irradiated with γ-rays under a reduced oxygen concentration and incubated at 37° C and 39° C, are shown in Figures 5.5 and 5.7 respectively. There was no cell growth at 41° C.

For cells irradiated under a reduced oxygen concentration and incubated at 37° C (Figure 5.5), a decrease in radiosensitivity from cells irradiated under oxic conditions can be observed.

This decrease in radiosensitivity can also be seen in Figure 5.7 for cells which were irradiated under a reduced oxygen concentration and then incubated at 39° C. The combined effects of γ-irradiation under a reduced oxygen concentration followed by an elevated incubation temperature are dose-dependent and do not inhibit cellular growth to the same extend as under normal conditions.

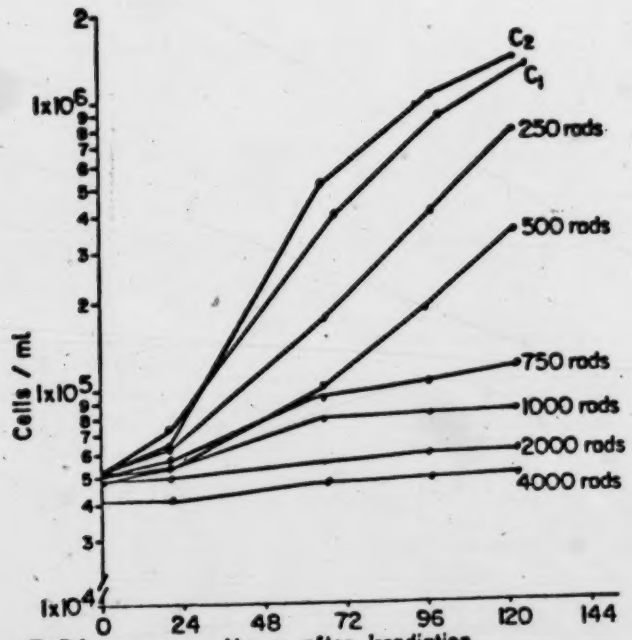


Fig.5.4 Growth of L1210 cells grown at 37°C after irradiation with γ rays.

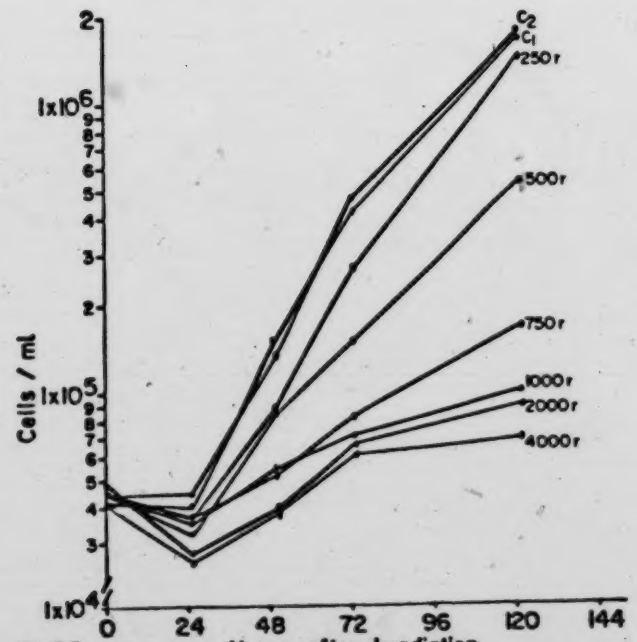


Fig.5.5 Growth of L1210 cells grown at 37°C after irradiation under reduced atmospheric oxygen concentration.

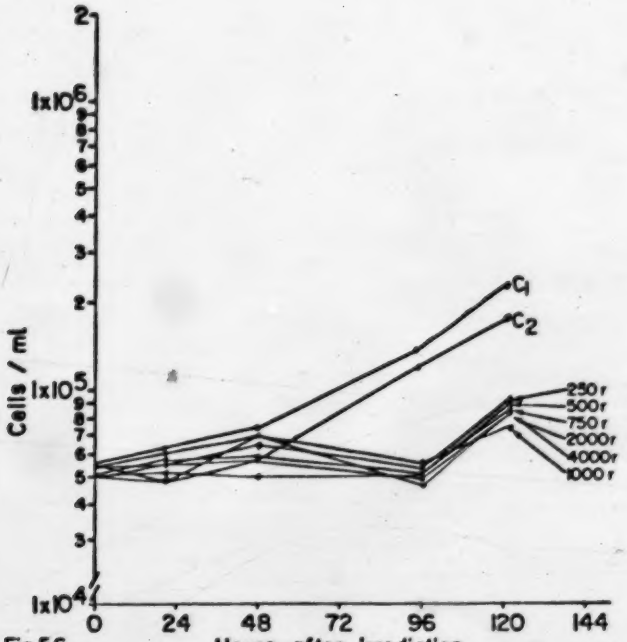


Fig. 5.6 Growth of L1210 cells grown at 39°C after irradiation with γ rays.

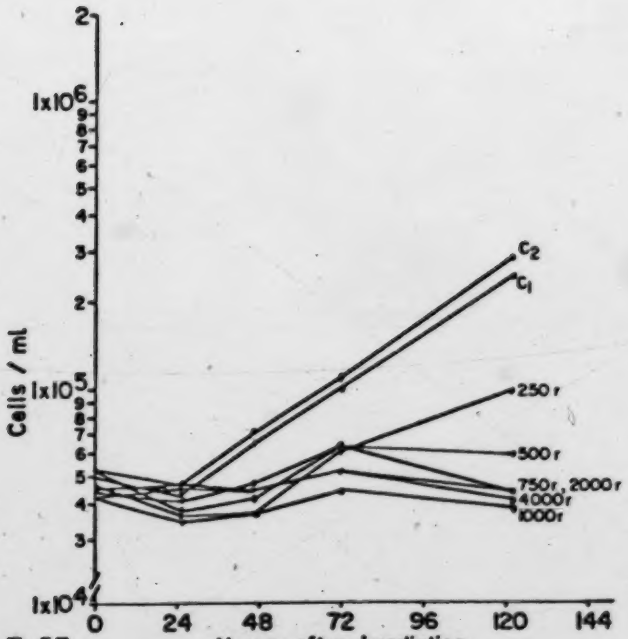


Fig. 5.7 Growth of L1210 cells grown at 39°C after irradiation under reduced atmospheric oxygen concentration.

Irradiation of L1210 cells under reduced and normal atmospheric oxygen concentrations yields different growth patterns. This indicates that a chemical agent (e.g., the presence or absence of oxygen) can perturb the cellular environment to a sufficient degree causing variations in cell growth. Standard deviation for this series of experiments is approximately 5%.

5.2.2 Growth Curves of γ-Irradiated Cultures

A growth curve for cells given varying doses of γ-radiation shown in Figure 5.8 (also Figure 5.5). The control sample began growing almost immediately whereas the irradiated cell suspensions experienced a mitotic delay which is longer with increasing dose. The number of cells which attain normal growth as compared to the control cells decreases as a function of increasing dose. Cells which received doses of 250 and 500 rads obtained at least one cycle of normal growth although they never reached a maximum concentration in suspension. Cells which received doses of 1000 and 2000 rads never reattained growth control. The reproductive ability of the cells was inhibited even though the cells were not killed directly. Cells which received a dose of 4000 rads were killed directly by lysing or had their reproductive ability impaired. Thus, a combination of reproductive death and actual cell death occurred. Giant cells were observed in cell cultures approximately 36 hours after irradiation but did not appear to be very numerous. These were also noted as abortive colonies in soft agar.

5.2.3 Growth Curves of Cells Exposed to Microwaves and Hyperthermia

Growth curves for cells exposed to 2450 MHz microwaves for periods of time up to one hour are shown in Figure 5.9. The initial cell

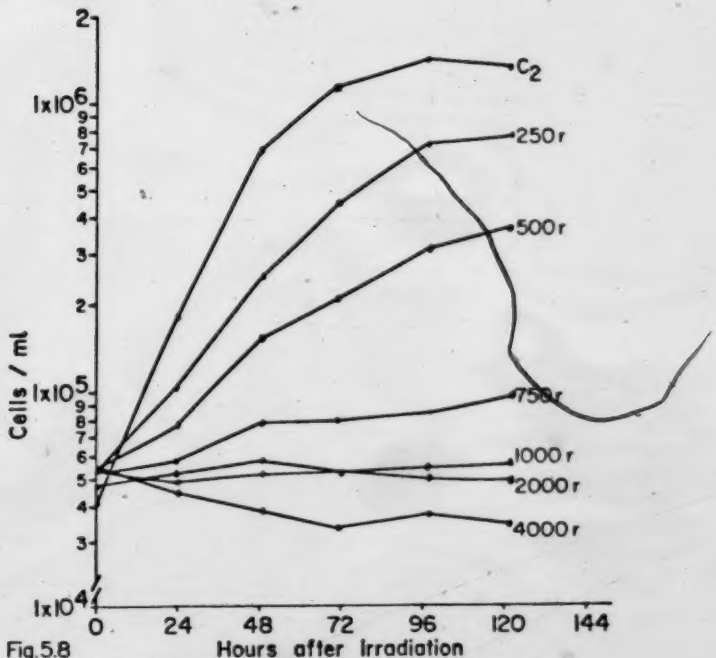


Fig. 5.8 Growth of L1210 cells irradiated with various doses of γ rays.

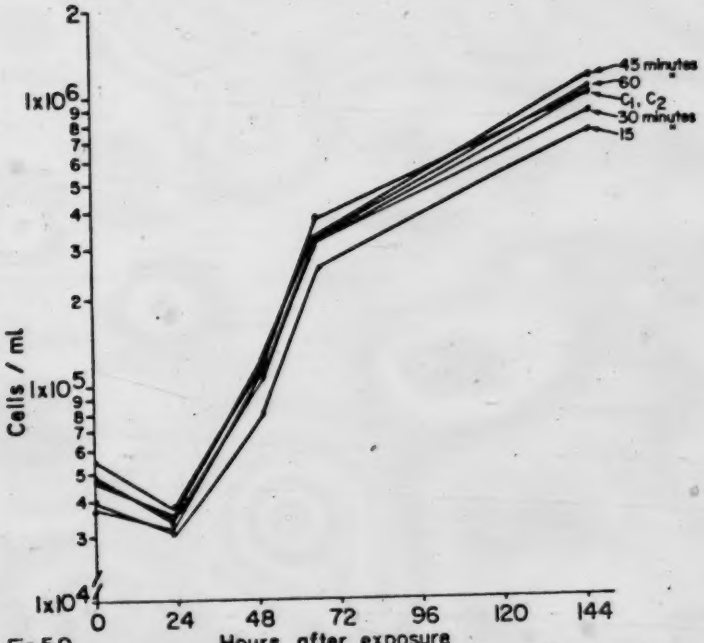


Fig. 5.9 Growth of cells exposed to 2450 MHz. microwaves for varying periods of time.

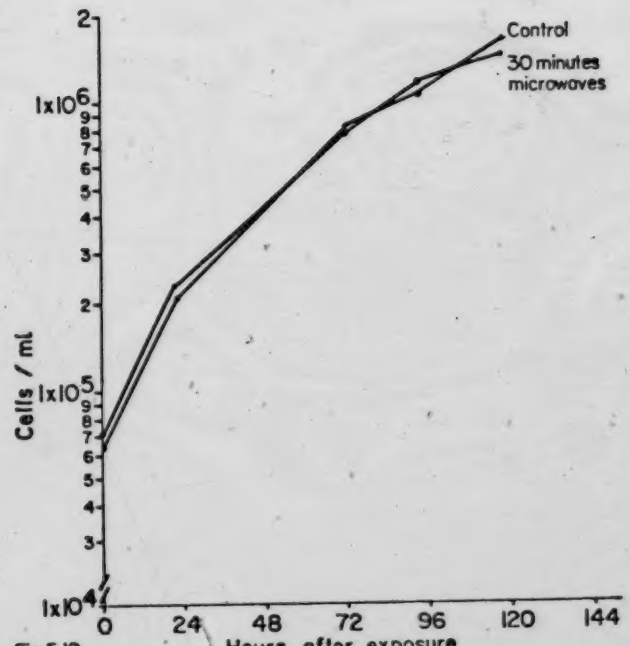


Fig. 5.10 Growth of cells exposed to 2450 MHz. microwaves.

temperature was 36.9° C. The two control cultures were left at room temperature for one hour. At the end of one hour, the final temperature was 22.7° C. After 15 minutes in the waveguide, the cell suspension had also reached a temperature of 22.7° C and maintained this throughout the microwave exposure. It is apparent from the graph that exposure of L1210 cells to 2450 MHz microwaves with an incident power of 100 mW for periods of time up to one hour does not have a significant effect on the reproductive ability of the cells. There is a mitotic delay evident, but since the control curves also exhibit this lag in cell growth, it can be assumed that it is a function of cell growth and concentration before dilution into fresh medium and not an actual mitotic delay due to irradiation.

Figure 5.10 compares a cell culture exposed to 2450 MHz microwaves for 30 minutes with a control culture. Initial temperature was 37.1° C; the final temperature was 23.4° C. There was no mitotic delay and the cell growth in both cases closely paralleled each other.

Since this series of experiments is designed to investigate non-thermal microwave effects, it is necessary to prevent heating of the cells by the microwaves. Cell temperature was closely monitored during microwave exposure. In all cases, cell samples, initially at the incubation temperature of 37° C, cooled down to room temperature (22.5-23.5° C) during the experiment. This temperature decrease paralleled that of control cultures which were placed in petri dishes and allowed to sit at room temperature for the duration of the experiment. Prior to microwave irradiation, a second control was obtained by diluting a small sample of the cells into fresh medium and placing it immediately in the incubator

at 37° C. For all microwave exposures of 15-60 minutes, there was no discernible difference in growth between control and microwave-exposed cell cultures.

To ensure that no cell heating had occurred, cell samples that were exposed only to microwaves were compared with cell samples which had been exposed to slight hyperthermia for the same length of time. Cell samples were placed in a 40° C water bath for 15-60 minutes. Within 15 minutes after being placed in the water bath, the cells had attained a temperature of 39.5° C. Final temperature was recorded as 39.9° C. The graphs in Figures 5.11 through 5.14 compare cell cultures which were exposed to either microwaves or hyperthermia (40° C) for 15, 30, 45, or 60 minutes. The pattern of effect is the same in all cases. Microwave exposure does not affect cellular growth at a frequency of 2450 MHz with an incident power of 100 mW for exposure times up to one hour. However, with hyperthermia there is an increase in mitotic delay and a decrease in the number of cells which reattain normal growth after hyperthermia. The relative number of cells which cannot replicate increases as a function of the exposure time of 40° C. In other words, cell death increases as the time of hyperthermic exposure increases for exposure times up to one hour. Experimental error for these experiments is approximately ±10%.

5.2.4 Growth Curves for Combinations of γ-Ray and Microwave Exposures

In order to determine whether low intensity microwaves have a nonthermal cellular effect, various experiments using different combinations of γ-ray and microwave exposures were carried out. It has been found that with in vitro cell culture techniques, variations between

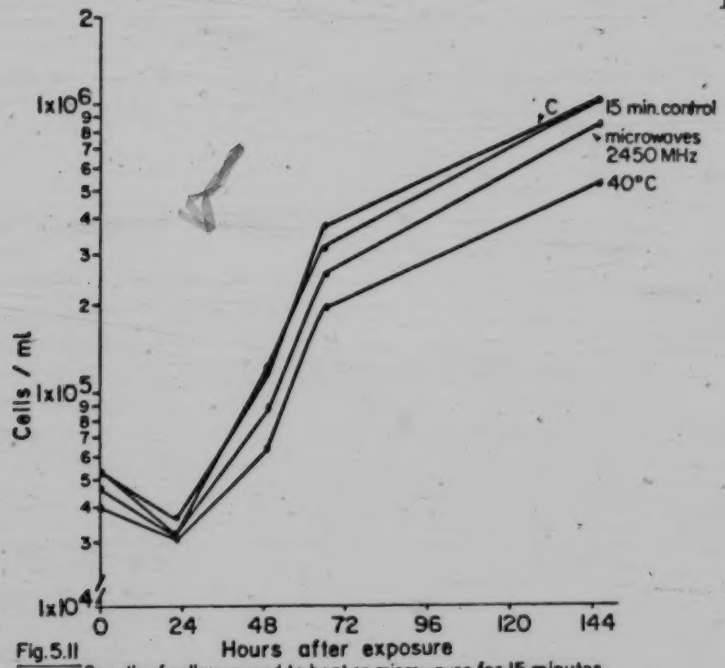


Fig. 5.11 Growth of cells exposed to heat or microwaves for 15 minutes.

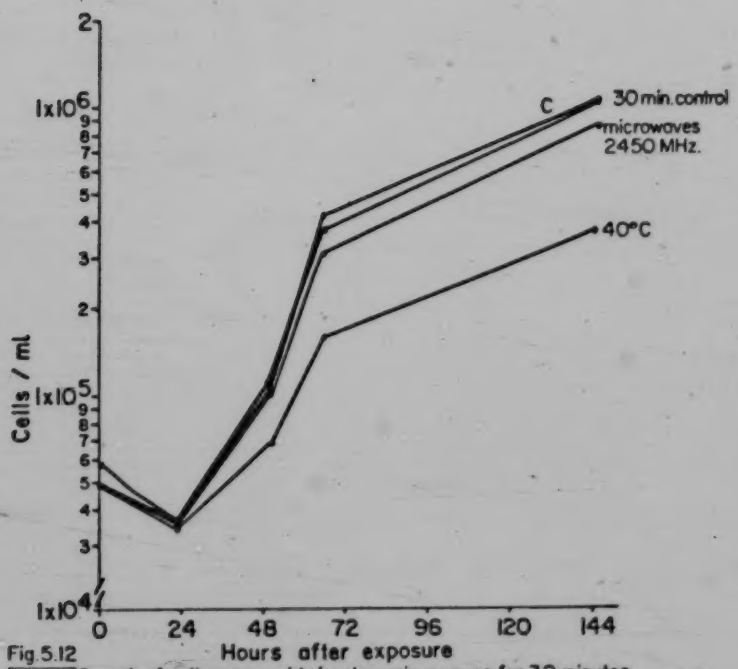


Fig. 5.12 Growth of cells exposed to heat or microwaves for 30 minutes.

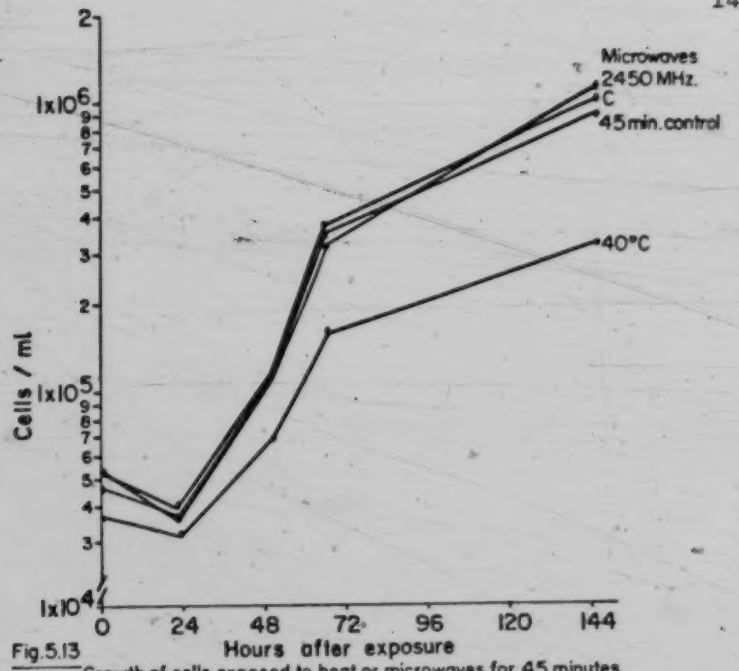


Fig. 5.13 Growth of cells exposed to heat or microwaves for 45 minutes

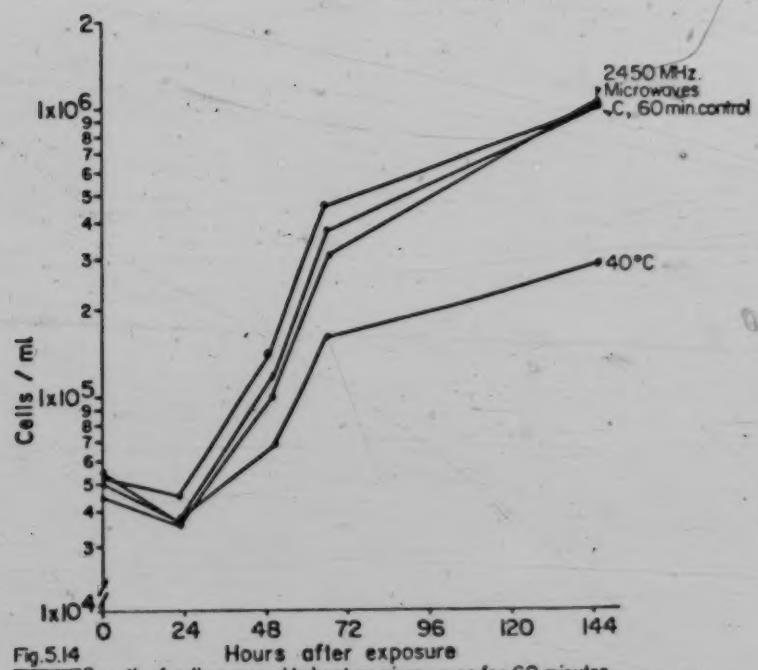


Fig. 5.14 Growth of cells exposed to heat or microwaves for 60 minutes.

successive experiments are much greater than within one experiment (Harisiadis et al., 1978). For this reason, experiments using various combinations of microwave and γ-radiation were done in large groups. It is more meaningful to compare relative magnitudes from any one experiment than to compare absolute magnitudes from different experiments. Figures 5.15 through 5.18 show the results of combined exposure of L1210 cells to microwaves and γ-radiation.

Figure 5.15 shows the effect of simultaneous microwave and γ-radiation. For each of the four γ-ray doses, 250, 500, 1000, and 2000 rads, the combined microwave and γ-ray curve closely parallels the γ-ray curve for that dose. This indicates similar growth of cell cultures. The mitotic delay of each of the four γ-ray curves is approximately the same as that of the microwave and γ-ray curve for the same dose of γ-radiation. This indicates that no combined response occurs with the radiation parameters involved.

In Figure 5.16, results of another experiment of the simultaneous irradiation of cells with microwaves and γ-rays are shown. The results are quite similar to those shown in Figure 5.17. Again, at each γ-ray dose, the growth curve closely parallels that of simultaneous microwave and γ-irradiation for the same γ-ray dose. In this case, however, the cells were exposed to 15 minutes of microwave exposure prior to beginning simultaneous microwave and γ-irradiation. This experiment was repeated many times and in each case there was no discernible difference between the effect of γ-rays on the cells and the combined effect of microwave and γ-ray exposure.

The experimental results shown in Figures 5.16 and 5.17 were obtained simultaneously and may be directly compared. In Figure 5.16

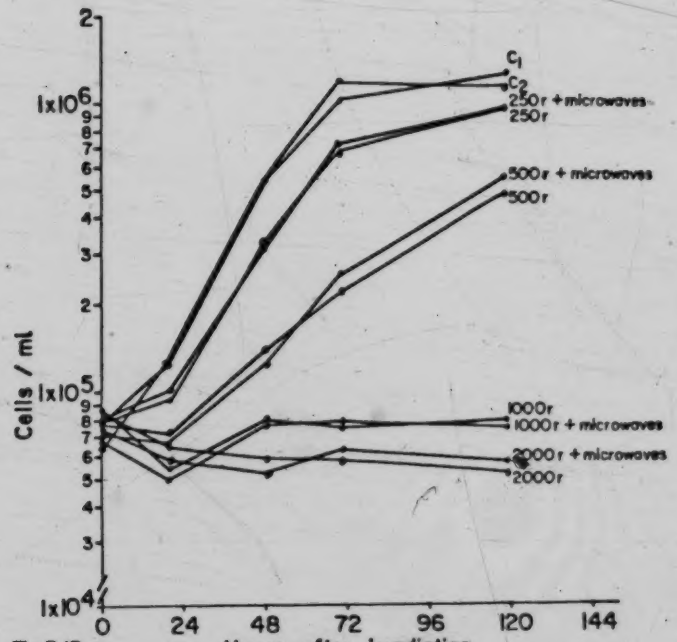


Fig.5.15 Growth of cells after simultaneous γ ray & microwave exposure.

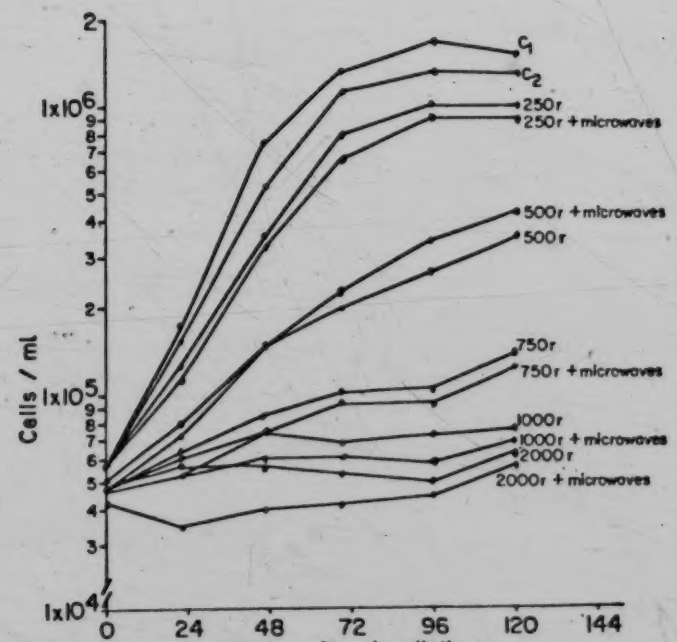


Fig.5.16 Growth of cells after simultaneous γ ray & microwave exposure.

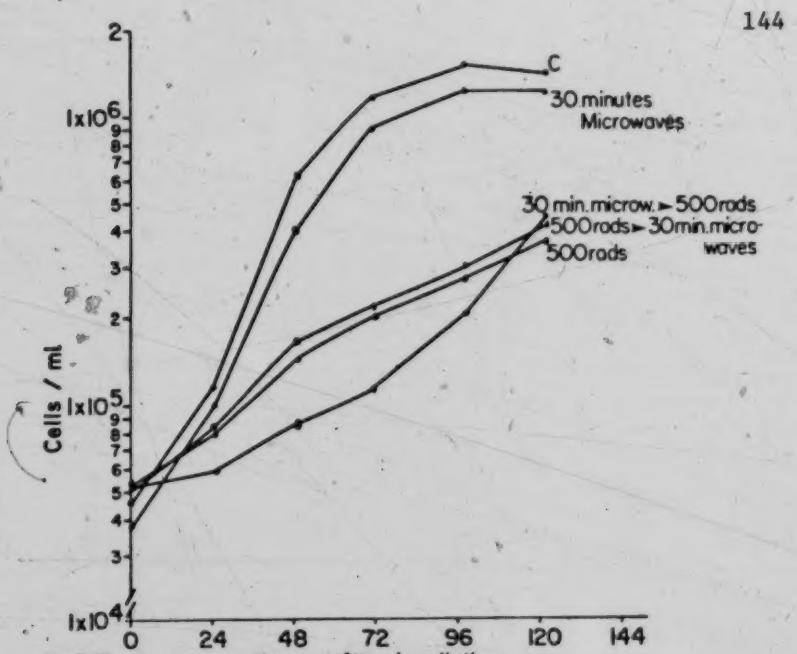


Fig.5.17 Growth of cells exposed to microwaves before & after γ radiation.

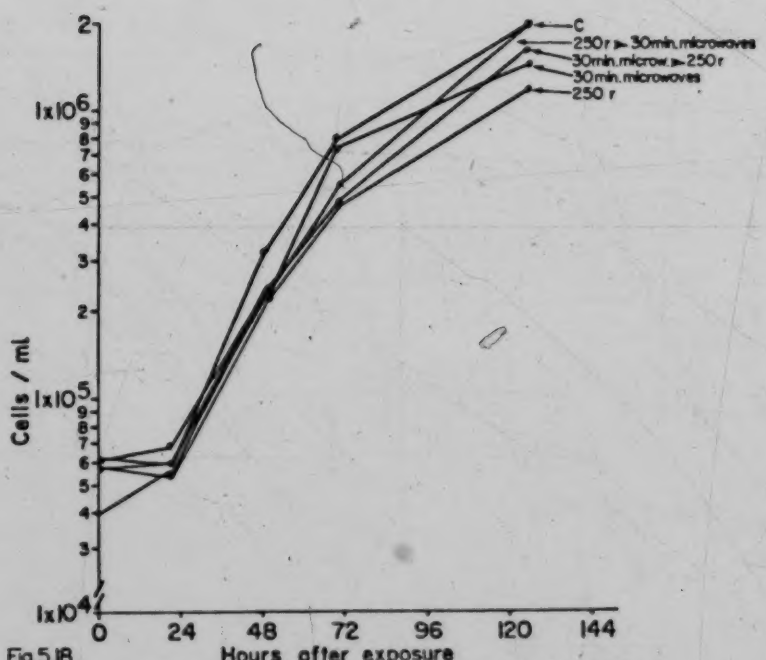


Fig.5.18 Growth of cells exposed to microwaves before & after γ radiation.

the control curve shows the same response as that of cells exposed to 30 minutes of 2450 MHz microwaves. This indicates that low intensity microwaves do not affect cellular growth. For cells exposed to microwaves before and after γ-radiation, the growth curves are nearly the same (within experimental error) as that for γ-radiation alone.

This experiment was repeated using a 30 minute microwave exposure with a 250 rad dose of γ-rays (Figure 5.18). In this case, all growth curves were similar. The growth curves of microwave exposure before and after γ-irradiation had the same mean generation times, mitotic delays, and grew to maximum cell concentration. The cell suspension which received 250 rads of γ-radiation did not grow to the same degree as the control cultures, whereas the two combined exposures were intermediate in growth. The differences in cell growth, however, were not significant. Subsequent experiments using microwave exposure before and after γ-irradiation showed that the longer mitotic delay as evidenced by cells exposed to microwaves prior to γ-irradiation did in fact exist to some degree but is not conclusive.

The results of these experiments indicate that low intensity microwaves do not significantly affect the growth of L1210 cells under the experimental conditions used.

5.2.5 Survival Curves

Colony-forming ability was used as an index of cell survival for mouse leukemia (L1210) cells exposed to γ-rays alone, and to simultaneous microwave and γ-radiation.

After a specific treatment, known but different numbers of cells were inoculated into test tubes. After an incubation period of 12-14 days,

the colonies were counted. Even in the control cultures, however, not all cells grew into viable colonies. The plating efficiency ranged from 70 to 75% in these experiments. This data was obtained by making several dilutions of control cells and plating different numbers of cells. Plating efficiency was determined by plotting incubation number against the number of colonies or clones which were formed, and determining the slope of the graph. This is shown in Figure 5.19.

When counting colonies, it is often difficult to distinguish colonies which have lost their reproductive ability. All cells which formed colonies, regardless of the size of the colony, were counted.

The γ-ray survival curve is shown in Figure 4.2. Evident from the curve are: a shoulder, indicating repair of sublethal damage; extrapolation number of 2.0; and exponential survival for doses from 100 to 600 rads. The dose (D_o) required to reduce cell survival by the factor 1/e (0.37) is determined from Figure 4.2 to be D_o = 190 rads. The quasi-threshold dose (D_q) is an indication of the width of the shoulder (Alper, et al., 1962). D_q depends on D_o and n by the relation $D_q = D_o \ln n$ (Elkind and Whitmore, 1967). D_q for L1210 cells is D_q = 130 rads.

The L1210 survival curve for simultaneous microwave and γ-irradiation is shown in Figure 5.20 along with a control survival curve for γ-rays alone. From the graph, it can be seen that there is no significant difference between the survival response of γ-rays and simultaneous microwave and γ-irradiation. Thus, the survival curve parameters are identical for both cases.

This dose-response curve indicates that low intensity microwaves do not perturb the cellular environment sufficiently to increase the

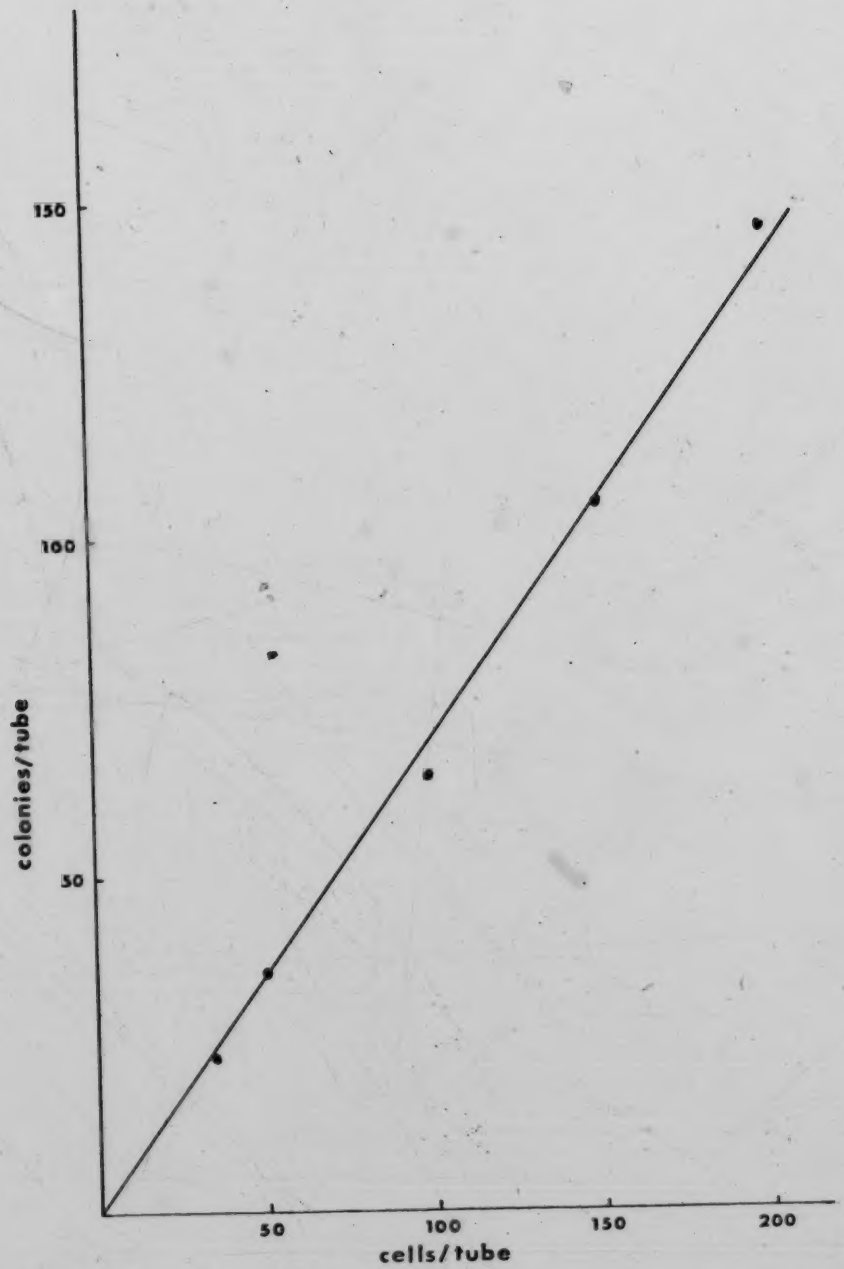


Fig. 5.19 Plating efficiency of colony-forming ability for L1210 cells.

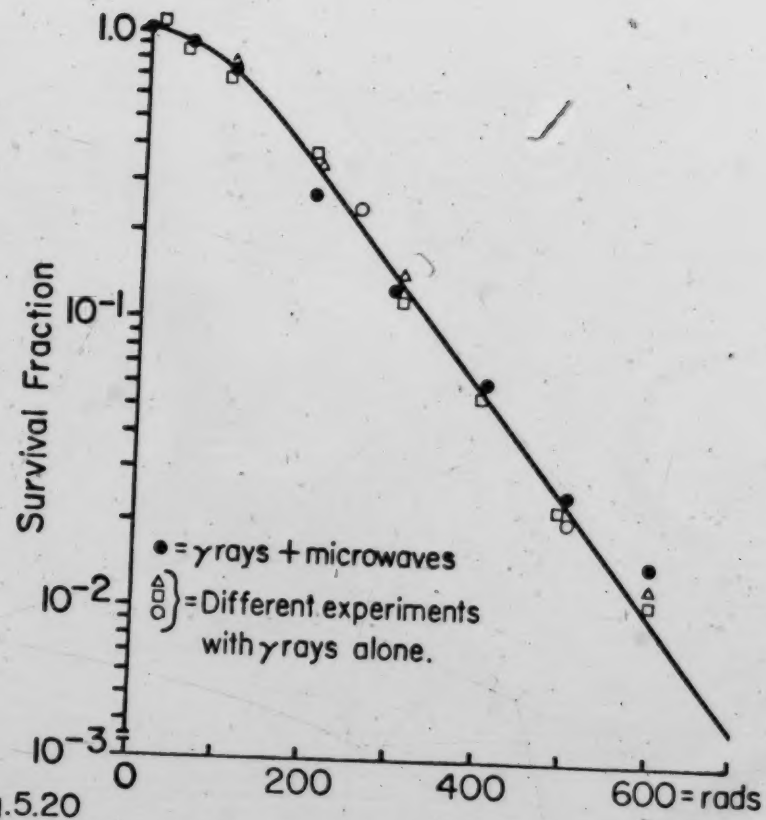


Fig.5.20 Survival of L1210 cells after simultaneous microwave & γ ray exposure compared with γ ray survival.

number of lethal or sublethal radiation events. This corroborates the findings of section 5.2.4 in which simultaneously applied microwaves did not alter the growth response of L1210 cells to γ-radiation.

CHAPTER VI

CONCLUSION AND DISCUSSION

The purpose of this study was to investigate the existence of nonthermal cellular microwave effects. In order to determine whether low intensity microwaves perturb the cellular environment, cells were exposed to combinations of microwave and γ-radiation. Both cellular growth and survival characteristics were used as indices to monitor the radiation response. Since cellular growth characteristics are a sensitive index of radiation response, microwaves which interact significantly with cells would be expected to alter the cell growth. Growth modification can be determined from both growth and survival curves.

In order to establish the normal growth pattern of mouse leukemia (L1210) cells, control cultures were routinely maintained. These controls were used as a basis for comparison with cell cultures which had been exposed to either or both ionizing and non-ionizing radiation. The control growth patterns remained constant over a time interval of two years (approximately 100 experiments).

Cell suspensions were exposed to 2450 MHz microwaves for periods up to one hour. In order to determine the magnitude of a cellular microwave effect, it is necessary to quantitate the exposure with the absorbed dose which produces the given effect. As discussed in section 5.1.3 (A), it is difficult to measure the absorbed power associated with low intensity microwave fields. For this reason, the absorbed power density in the cell suspension was determined using a technique developed

by Courtney et al. (1975). The absorbed power density in the cell suspension was calculated to be approximately 1 mW/cm^2 corresponding to a specific absorption rate of 2.0 mW/g. The temperature of the sample was closely monitored during the microwave exposure to determine if this specific absorption rate would cause a temperature increase. Exposure to microwave radiation for periods of time up to one hour did not result in an overall increase in temperature. Since the cell suspensions were irradiated at room temperature (22.5° C), any heat was quickly dissipated as the sample cooled down from the incubation temperature of 37° C. The limiting factor in these experiments was the inability of the cells to tolerate sub-optimum environmental conditions for any length of time (e.g., fluctuation of pH from the normal of approximately 7.2, temperature deviation from 37° C). As such, each experiment was confined to a time period of one hour.

The growth curves of cells exposed to microwaves for time intervals up to one hour did not differ significantly from control growth curves. Low intensity microwaves at a frequency of 2450 MHz did not alter the normal growth of L1210 cells.

Similarly, cells which were exposed to microwaves before, after, or during γ-irradiation, did not differ in cell growth or survival from the controls. This indicates that microwave radiation under the experimental conditions discussed in section 5.1.3, does not magnify, or alter, the cellular response due to γ-irradiation.

It is very difficult to relate experiments with cellular systems in vitro to those in vivo. There are differences in the physical environment such as pH, ion concentration, and oxygen tension. Cells in vitro may be found in aqueous or non-aqueous solutions, leading to

inherent differences in radiosensitivity. Cells in aqueous solutions may be grown in suspension cultures or monolayers; cells in vivo may in fact be found in various complexes or aggregates. As well, each species is different in terms of the relative sensitivity of various tissues and organs to microwaves. For this reason alone, it is difficult to apply experimental results of microwave absorption from in vitro to in vivo cellular systems. However, if the mechanism for nonthermal cellular effects can be determined, this knowledge may be applied to nonthermal microwave effects which occur in vivo.

It has already been shown that cultured mammalian cells may be altered by the use of various chemical and physical agents before, during, or after exposure to ionizing radiation (Giovanella et al., 1970; Ben-Hur et al., 1972; Cass and Au-Yeung, 1976; Harisiadis et al., 1978). Hyperthermia induced by high intensity microwave radiation has been shown to alter cellular radiation response (Streffer et al., 1978). However, the mechanism for the synergistic effect has not been determined.

Hyperthermic sensitization of cells to ionizing radiation may be due solely to the effect of an increase in temperature or there may be an underlying microwave interaction which is masked by the hyperthermia. The threshold at which hyperthermia is induced depends on the microwave intensity and duration, and on the absorption characteristics of the biological system. As the microwave exposure is reduced to a point where hyperthermia is not induced, it remains to be proven whether microwaves and ionizing radiation still exert a synergistic effect on the cells.

As a first attempt to determine the mechanism of a nonthermal cellular effect, this study has applied low intensity microwaves at a

frequency of 2450 MHz to cell systems. The results of this study have shown that L1210 cells do not exhibit such a synergistic effect at low power densities (approximately 1 mW/cm^2). Within experimental limitations, it was not possible to determine the threshold for a synergistic effect.

The mechanism by which synergistic microwave effects occur must be clearly understood before microwave exposure can be used clinically in conjunction with normal radiotherapy treatment.

BIBLIOGRAPHY

Adams, G. E. 1970. Molecular mechanisms of cellular radiosensitization and protection. In: Radiation Protection and Sensitization, H. L. Moroson and M. Quintiliani, Eds. Taylor and Francis, Ltd., London, pp. 3-14.

Alexander, P. 1960. Mouse lymphoma cells with different radiosensitivities. Nature 192:572.

Alexander, P., and Charlesby, A. 1954. Energy transfer in molecules exposed to ionizing radiation. Nature 173:578-579.

Alexander, P., and Charlesby, A. 1955. Radiobiology Symposium Proceedings, Liege, Butterworth, London, p. 49.

Alper, T., Fowler, J. F., Morgan, R. L., Vonberg, D. D., Ellis, F., and Oliver, R. 1962. The characterization of the 'Type C' survival curve. Brit. J. Radiol. 35:722-723.

Altman, P. L., and Dittmer, D. S. 1961. Blood and Other Body Fluids, Federation of American Societies for Experimental Biology, Bethesda, Md.

Anne, A., Saito, M., Salati, O. M., and Schwan, H. P. 1961. Relative microwave absorption cross sections of biological significance. Proceedings of the 4th Annual Tri-Service Conference on Biological Effects of Microwave Radiating Equipments: Biological Effects of Microwave Radiations, M. F. Peyton, Ed. Plenum Press, New York.

Attix, F. H., Roesch, W. C., and Tochilin, E. (Eds.). 1968. Radiation Dosimetry, Vol. I Fundamentals. Academic Press, New York.

Bacchetti, S., and Sinclair, W. K. 1971. The effects of X-rays on the synthesis of DNA, RNA, and proteins in synchronized Chinese hamster cells. Radiat. Res. 45:598.

Bacq, Z. M., and Alexander, P. 1961. Fundamentals of Radiobiology, 2nd Ed. Pergamon Press, Oxford.

Baranski, S., and Czerski, P. 1976. Biological Effects of Microwaves. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pennsylvania.

Baranski, S., Szmigielski, S., and Moneta, J. 1974. Effects of microwave irradiation in vitro on cell membrane permeability. In: Biological Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Proceedings of an International Symposium, Warsaw, 15-18 October 1973. Polish Medical Publishers, pp. 173-175.

Barber, D. E. 1962. The reaction of luminous bacteria to microwave radiation in the frequency range of 2608.7-3082.3 Mc. Technical Report, University of Michigan School of Public Health, IRE Trans. BME-9:77-80.

Barnes, F. S., and Hu, C. 1977. Model for some nonthermal effects of radio and microwave fields on biological membranes. IEEE Trans. of Microwave Theory and Techniques MTT 25(9):42-746.

Ben-Hur, E., Bronk, B. V., and Elkind, M. M. 1972. Thermally enhanced radiosensitivity of cultured Chinese hamster cells. Nature (New Biology) 238:209-211.

Ben-Hur, E., Elkind, M. M., and Bronk, B. V. 1974. Thermally enhanced radio-response of cultured Chinese hamster cells: Inhibition of repair of sublethal damage and enhancement of lethal damage. Radiat. Res. 58:38-51.

Ben-Hur, E., Elkind, M. M., and Riklis, E. 1978. The combined effects of hyperthermia and radiation in cultured mammalian cells. In: Cancer Therapy by Hyperthermia and Radiation, C. Streffer et al., Eds. Urban and Schwarzenburg, Baltimore, pp. 29-36.

Boag, J. W. 1967. The events following primary activations. Proceedings of the Third International Congress of Radiation Research, G. Silini, Ed. North-Holland Publishing, Amsterdam, pp. 43-53.

Braams, R. 1966. Rate constants of e^-_{aq} reactions with amino acids. Radiat. Res. 27:319.

Braams, R. 1967. The effects of ionizing radiations on proteins. Proceedings of the Third International Congress of Radiation Research, G. Silini, Ed. North Holland Publishing Co., pp. 371-383.

Braams, R., and Ebert, M. 1968. The influence of changes of conformation of a macromolecule on reaction rates. In: Radiation Chemistry, Vol. 1, Advances in Chemistry Series 81, R. F. Gould, Ed. ACS Publications, Washington, p. 464.

Brent, T. P., and Wheatley, G. A. 1971. Repair and replication of X-irradiated HeLa cell DNA. Int. J. Radiat. Biol. 19:339.

Bronk, B. 1976. Thermal potentiation of mammalian cell killing: Clues for understanding and potential for tumor therapy. In: Advances in Radiation Biology, Vol. 6. Academic Press, Inc., New York, pp. 267-324.

Burton, M., and Magee, J. (Eds.). 1969. Advances in Radiation Chemistry, Vol. I. Wiley Interscience, New York.

Buxton, G. V. 1968. Primary radical and molecular yields in aqueous solution; the effect of pH and solute concentration. Radiat. Res. Rev. 1:209-222.

Carpenter, R., Biddle, D. K., and van Ummersen, C. A. 1960. Opacities in the lens of the eye experimentally induced by exposure to microwave radiation. Trans. IRE. Med. Electronics ME-7:152.

Carpenter, C. M., and Page, A. B. 1930. The production of fever in man by short radio waves. Science 71:450.

Casarett, A. P. 1968. Radiation Biology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Cass, C. E., and Au-Yeung, T. H. 1976. Enhancement of 9-β-D-Arabinofuranosyladenine cytotoxicity to mouse leukemia L1210 in vitro by 2'-deoxycoformycin. Cancer Res. 36:1486-1491.

Cavaliere, R., Ciocatto, E. C., Giovanella, B. C., Heidleberger, C., Johnson, R. O., Margottini, M., Moricca, G., and Rossi-Fanelli, A. 1967. A selective heat sensitivity of cancer cells. Cancer 20: 1351-1381.

Chen, K. C., and Lin, J. C. 1978. A system for studying effects of microwaves on cells in culture. J. Microwave Power 13(3):251-256.

Chernavskii, D. S., et al. 1967. Elastic deformations of proteins. Proceedings of Symposium on Physics and Biology, Moscow, p. 4.

Clark, F. W. 1950. Effects of intense microwave radiation on living organisms. Proc. IRE 38:1028-1032.

Cleary, S. F. 1970. Biological effects of microwave and radiofrequency radiation. CRC Critical Reviews in Environmental Control 1:257-302.

Cleary, S. F. 1973. Uncertainties in the evaluation of the biological effects of microwave and radiofrequency radiation. Health Physics 25:387-404.

Cleary, S. F. 1977. Biological effects of microwave and radiofrequency radiation. CRC Critical Reviews in Environmental Control, pp. 121-166.

Cleary, S. F., and Pasternock, B. S. 1966. Lenticular changes in microwave workers. Arch. Environ. Health 12:23-29.

Cole, A., Cooper, W. G., Shonka, F., Corry, P. M., Humphrey, R. M., and Ansevin, A. T. 1974. DNA scission in hamster cells and isolated nuclei studied by low voltage electron beam irradiation. Radiat. Res. 60:1-33.

Collinson, E., Dainton, F. S., and Holmes. B. 1950. Inactivation of ribonuclease in dilute aqueous solutions: Inactivation by hydroxyl radicals. Nature 165:267.

Collyns, B., Okada, S., Scholes, G., Weiss, J. J., and Wheeler, C. M. 1965. Chain scission and H bond breakage on irradiation of DNA. Radiat. Res. 25:526.

Cook, H. F. 1952. A physical investigation of heat production in human tissues when exposed to microwaves. Brit. J. Appl. Phys. 3:1-6.

Cook, H. F. 1952. The pain threshold for microwave and infrared radiations. J. Physiol. 118:1-11.

Cooper, R. D., and Wood, R. W. 1974. Physical Mechanisms in Radiation Biology, U.S. Atomic Energy Commission Division of Biomedical Research, Conference Proceedings, Airlie, Va., 11-14 October, 1972.

Courtney, K. C., Lin, J. C., Guy, A. W., and Chou, C. K. 1975. Microwave effects on rabbit superior cervical ganglion. IEEE Trans. MTT-23(10):809-813.

Crile, G. 1963. The effects of heat and radiation on cancers implanted on the feet of mice. Cancer Res. 23:372-380.

Crowther, J. A. 1924. Action of x-rays on tissue cells. Proc. Roy. Soc. B. 96:207.

Czerski, P. 1974. Influence of microwave radiation on the hematopoietic system. In: Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Polish Medical Publishers, Warsaw.

Czerski, P. 1975. Experimental models for the evaluation of microwave biological effects. Proc. IEEE 63(11):1540-1544.

Czerski, P. 1975. Microwave effects on the blood forming system with particular reference to the lymphocyte. In: Biologic Effects of Nonionizing Radiation, P. E. Tyler, Ed. Ann. N.Y. Acad. Sci. 247: 232-242.

Czerski, P., Ostrowski, K., Shore, M. L., Silverman, C., Suess, M. J., and Waldeskog, B. (Eds.). 1974. Biologic Effects and Health Hazards of Microwave Radiation, Proceedings of an International Symposium, Warsaw, 15-18 October, 1973. Polish Medical Publishers.

Daily, L. E. 1943. A clinical study of the results of exposure of laboratory personnel to radar and high frequency radiation. U.S. Navy Bulletin 41:1052-1065.

Dainton, F. S. 1967. Introductory paper. In: The Chemistry of Ionization and Excitation, G. R. A. Johnson and G. Scholes, Eds. Taylor and Francis Ltd., London, pp. 3-15.

Deficis, A., et al. 1973. Biological alterations observed under microwave irradiation. Proceeding of the Microwave Power Symposium, University of Waterloo, 1975, pp. 48-58.

Dertinger, H., and Jung, H. 1970. Molecular Radiation Biology. Springer-Verlag, New York.

Dewey, W. C., Hopwood, L. E., Sapareto, S. A., and Gerweck, L. E. 1977. Cellular responses to combinations of hyperthermia and radiation. Radiology 123:463-474.

Dewey, W. C., Humphrey, R. M., and Cork, A. 1963. Comparison of cell multiplication and colony formation as criteria for radiation damage in cells grown in vitro. Int. J. Radiat. Biol. 6:463.

Dewey, W. C., Noel, J. S., and Dettor, C. M. 1972. Changes in radiosensitivity and dispersion of chromatin during the cell cycle of synchronous Chinese hamster cells. Radiat. Res. 52:373.

Dickson, J. A., and Shah, D. M. 1972. The effects of hyperthermia (43°C) on the biochemistry and growth of a malignant cell line. Eur. J. Cancer 8:561-568.

Dickson, J. A., and Shah, S. A. 1977. Technology for the hyperthermic treatment of large solid tumours at 50°C. Clin. Oncol. 3:310-318.

Dickson, J. A., Shah, S. A., Waggott, D., and Whalley, W. B. 1977. Tumor eradication in the rabbit by radiofrequency heating. Cancer Res. 37:2162-2169.

Dixon, G., Dulmadge, E., and Schabel, F. 1966. Growth requirements and biologic characteristics of leukemia L1210 cells in culture. Cancer Chemotherapy Reports 50(5):247-254.

Dodge, C. H. 1970. Clinical and hygienic aspects of exposure to electromagnetic fields. In: Biologic Effects and Health Implications of Microwave Radiation, Symposium Proceedings, USDHEW, Rep. PHS, BRH, DBE 70-2, p. 140.

Doida, Y., and Okada, S. 1969. Radiation induced mitotic delay in cultured mammalian cells (L5178Y). Radiat. Res. 38:513.

Durand, R. E. 1978. Potentiation of radiation lethality by hyperthermia in a tumor model: Effects of sequence, degree, and duration of heating. Int. J. Rad. Oncol. Biol. Phys. 4:401-405.

Ebert, M. 1970. Damaging radiation chemical events. In: Radiation Protection and Sensitization, H. L. Moroson and M. Quintiliani, Eds. Taylor and Francis, Ltd., London, pp. 133-136.

Elkind, M. M. 1967. Sub-lethal X-ray damage and its repair in mammalian cells. In: Proceedings of the Third International Congress of Radiation Research, G. Silini, Ed. North-Holland Publishing, Amsterdam, pp. 558-586.

Elkind, M. M., and Sutton, H. 1959. X-ray damage and recovery in mammalian cells in culture. Nature 184:1293-1295.

Elkind, M. M., and Sutton, H. 1960. Radiation response of mammalian cells grown in culture. I. Repair of x-ray damage in surviving Chinese hamster cell. Radiat. Res. 13:556-593.

Elkind, M. M., Sutton-Gilbert, H., Moses, W. B., and Kamper, C. 1967. Sub-lethal and lethal radiation damage. Nature 214:1088.

Elkind, M. M., and Whitmore, G. F. 1967. The Radiobiology of Cultured Mammalian Cells. Gordon and Breach, New York.

Evans, Robley D. 1955. The Atomic Nucleus. McGraw-Hill Book Company, Inc., New York.

Fielden, E. M., and Lillicrap, S. C. 1972. Excited states and energy transfer in biomolecular systems. In: Current Topics in Radiation Research, Vol. 7, Ebert and A. Howard, Eds. North-Holland Publishing, Amsterdam, pp. 132-180.

Finney, D. J., Hazlewood, T., and Smith, M, J. 1955. Logarithms to base 2. J. Gen. Microbiol. 12:222-225.

Fischer, G. A., and Sartorelli, G. P. 1964. Development, maintenance and assay of drug resistance. Meth. Med. Res. 10:247.

Fitzgerald, J. J., Brownell, G. L., and Mahoney, F. J. 1967. Mathematical Theory of Radiation Dosimetry. Gordon and Breach Science Publishers, Inc., New York.

Fleming, H. 1944. Effects of high frequency fields on micro-organisms. Electrical Engineering 63:18-21.

Fleming, J., et al. 1961. Microwave radiation in relation to biological systems and neural activity. In: Biological Effects of Microwave Radiation, M. F. Peyton, Ed. Plenum Press, New York, p. 239.

Frey, A. H. 1962. Human auditory system response to modulated electromagnetic energy. J. Appl. Physiol. 1:689-692.

Frey, A. H. 1971. Biological function as influenced by low-power modulated RF energy. IEEE Trans. on Microwave Theory and Techniques MTT-19(2):134-164.

Frohlich, H. 1975. The extraordinary dielectric properties of biological materials and the action of enzymes. Proc. Natl. Acad. Sci. 72:4211-4215.

Freeman, G. R. 1973. Radiation Chemistry, Vols. I and II, Unpublished.

Gandhi, O. P. 1974. Polarization and frequency effects on whole animal absorption of RF energy. IEEE 62(8):1166-1168.

Gandhi, O. P. 1975. Frequency and orientation effects on whole animal absorption of electromagnetic waves. IEEE Trans. Biomed. Eng. Nov. 1975, pp. 536-542.

Garrison, W. M. 1964. Action of ionizing radiations on nitrogen compounds in aqueous solutions. Radiat. Res. Suppl. 4:158.

Garrison, W. M. 1968. Radiation chemistry of organo-nitrogen compounds. In: Current Topics in Radiation Research, Vol. 4, M. Ebert and A. Howard, Eds. North-Holland Publishing, Amsterdam, p. 43.

Garrison, W. M., and Weeks, B. M. 1962. Radiation chemistry of compounds containing the peptide bond. Radiat. Res. 17:341.

Gerweck, L. E., Gilette, E. L., and Dewey, W. C. 1975. Effect of heat and radiation on synchronous Chinese hamster cells: Killing and repair. Radiat. Res. 64:611-623.

Giovanella, B. C., Lohman, W. A., and Heidelberger, C. 1970. Effects of elevated temperatures and drugs on the viability of L1210 leukemia cells. Cancer Res. 30:1623-1631.

Giovanella, B. S., Morgan, A. C., Stehlin, J. S., and Williams, L. J. 1973. Selective lethal effect of supranormal temperatures on mouse sarcoma cells. Cancer Res. 33:2568-2578.

Glaser, Z. 1975. Sixth Supplement to Bibliography of Reported Biological Phenomena ('Effects') and Clinical Manifestations Attributed to Microwave and Radio-Frequency Radiation; EMR Project Office, Naval Medical Research and Development Command, July 1975 (NMRDC Rept. No. 3) (AD #A015-622). [5th Supplement, July 1974, AD #784-007; 4th Supplement, June 1973, AD #770-621; Revised Report (April 1972), containing 1st Supplement (October 1971), 2nd Supplement (November 1971), and 3rd Supplement (April 1972), AD #750-271; Original Report, October 1971, AD #734-391]

Gordon, Z. V., Roscin, A. V., and Bychov, M. S. 1974. Main directions and results of research conducted in the U.S.S.R. on the biologic effects of microwaves. In: Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Polish Medical Publishers, Warsaw.

Grant, E. H. 1974. Microwaves - A tool in medical and biological research. In: Biological Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Proceedings of an International Symposium, Warsaw, 15-18 October, 1973, Polish Medical Publishers, pp. 309-316.

Gray, L. H., Conger, A. D., Ebert, M., Hornsey, N., and Scott, O. C. A. 1953. Concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Brit. J. Radiol. 26:638-648.

Grodsky, I. T. 1975. Possible physical substrates for the interaction of electromagnetic fields with biological membranes. In: Biological Effects of Non-ionizing Radiation, P. Tyler, Ed. Ann. N.Y. Acad. Sci. 247:117-123.

Guy, A. W. 1971. Analyses of electromagnetic fields induced in biological tissues by thermographic studies on equivalent phantom models. IEEE Trans. Microwave Theory Techniques MTT-19:205-214.

Hahn, G. M. 1974. Metabolic aspects of the role of hyperthermia in mammalian cell inactivation and their possible relevance to cancer treatment. Cancer Res. 34:3117-3123.

Hardy, J. D. 1958. Thermal radiation, pain and injury. In: Therapeutic Heat, Vol. 2, S. Licht, Ed. New Haven, Conn., pp. 157-178.

Harisiadis, L., Sung, D., and Hall, E. J. 1977. Thermal tolerance and repair of thermal damage by cultured cells. Radiology 23:505-509.

Harisiadis, L., Sung, D., Kessaris, N., and Hall, E. J. 1978. Hyperthermia and low dose-rate irradiation. Radiology 129:195-198.

Har-Kedar, I., and Bleehan, N. 1976. Experimental and clinical aspects of hyperthermia applied to the treatment of cancer with special reference to the role of ultrasonic and microwave heating. In: Advances in Radiation Biology, Vol. 6. Academic Press Inc., New York, pp. 229-266.

Hayon, E. 1968. Primary radical yields in the radiation chemistry of water and aqueous solutions. In: Radiation Chemistry of Aqueous Systems, G. Stein, Ed. Weizmann Science Press, Jerusalem, pp. 157-209.

Healer, J. 1970. Review of studies of people occupationally exposed to radiofrequency radiation. In: Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings, S. F. Cleary, Ed. Richmond, Sept. 17-19 (BRH/DBE Rept. No. 70-2), pp. 90-97.

Heinmets, F., and Hershman, A. 1961. Consideration of the effects produced by superimposed electric and magnetic fields in biological systems and electrolytes. Phys. Med. Biol. 5:271.

Heitler, W. 1954. The Quantum Theory of Radiation, 3rd Ed. Oxford University Press, London and New York.

Heller, J. 1959. Effect of high-frequency electromagnetic fields on microorganisms. Radio Electronics 6:6.

Heller, J. H. 1970. Cellular effects of microwave radiation. In: Biologic Effects and Health Implications of Microwave Radiation, Symposium Proceedings, USDHEW, Rep. PHS, BRH, DBE 70-2, pp. 116-121.

Heller, J. H., and Teixeira-Pinto, A. A. 1959. A new physical method of creating chromosomal aberrations. Nature 183:905-906.

Hems, G. 1960. Effects of ionizing radiation on aqueous solutions of inosine and adenosine. Radiat. Res. 13:777.

Hendler, E. 1968. Cutaneous receptor response to microwave irradiation. In: Thermal Problems in Aerospace Medicine, J. D. Hardy, Ed. Surrey, England, pp. 149-161.

Hendler, E., and Hardy, J. D. 1958. Temporal aspects of temperature sensation. ASME Paper 58-A-220.

Hendler, E., and Hardy, J. D. 1960. Infrared and microwave effects on skin heating and temperature sensation. IRE Trans. Med. Electron. ME-7:143-152.

Hendler, E., Hardy, J. D., and Murgatroyd, D. 1963. Skin heating and temperature sensation produced by infra-red and microwave irradiation. In: Temperature Measurement and Control in Science and Industry, Part 3, Biology and Medicine, J. D. Hardy, Ed. Reinhold, New York, pp. 221-230.

Henley, E. J., and Johnson, E. R. 1969. The Chemistry and Physics of High Energy Reactions. University Press, Washington, D.C.

Herrick, J. 1958. Pearl chain formation. Proceedings of the Second Tri-Service Conference on Biological Effects of Microwave Energy, Rome. Tech. Rep. ARDC-1, TR-58-54, ASTIA Doc. 137-477, pp. 83-93.

Herrick, J. F., and Krusen, F. H. 1953. Certain physiologic and pathologic effects of microwaves. Elec. Eng. 72:239-244.

Hill, T. 1958. Some possible biological effects of an electric field acting on nucleic acids or proteins. J. Am. Chem. Soc. 8:2142.

Himmelfarb, P., Thayer, P. S., and Martin, H. 1967. Growth of colonies of murine leukemia L1210 in vitro. Cancer Chemotherapy Rept. 51(7): 451-458.

Hine, G. J., and Brownell, G. L. 1956. Radiation Dosimetry. Academic Press Inc., New York.

Hofer, K. G., Hofer, M. G., Ieracitano, J., and McLaughlin, W. H. 1977. Radiosensitization of hypoxic tumor cells by simultaneous administrations of hyperthermia and nitroimidazoles. Radiat. Res. 70:362.

Hopwood, L. E., and Tolmach, L. J. 1971. Deficient DNA synthesis and mitotic death in X-irradiated HeLa cells. Radiat. Res. 46:70.

Howland, J. W., and Michaelson, S. 1962. Studies on the biological effects of microwave irradiation on the dog and rabbit. In: Proceedings of the Third Annual Tri-Service Conference on Biological Hazards of Microwave Radiating Equipments, C. Susskind, Ed. Univ. of California, Berkeley, p. 191.

Howland, J., and Michaelson, S. 1964. The effect of microwaves on the biologic response to ionizing radiation. Ind. Med. Surg. 33:500.

Illinger, K. H. 1974. Interaction between microwave and millimeter-wave electromagnetic fields and biological systems: Molecular mechanisms. In: Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Proceeding of an International Symposium, Warsaw, 15-18 October 1973. Polish Medical Publishers, pp. 160-172.

International Commission on Radiological Units and Measurements. 1970. Linear Energy Transfer, Report 16. ICRU, Washington, D.C.

International Commission on Radiological Units and Measurements. 1971. Radiation Quantities and Units, Report 19. ICRU, Washington, D.C.

Johns, H. E. 1966. The Physics of Radiology. Charles C. Thomas, Springfield, Illinois.

Johnson, C. C. 1973. Research needs for establishing a radio frequency electromagnetic radiation safety standard. J. Microwave Power 8(3/4): 367-368.

Johnson, C., Durney, C., and Massoudi, H. 1975. Electromagnetic power absorption in anisotropic tissue media. IEEE Trans. on Microwave Theory and Techniques, Annals No. 506MT016, pp. 529-532.

Johnson, C. C., and Guy, A. W. 1972. Nonionizing electromagnetic wave effects in biological materials and systems. Proc. IEEE 60(6): 692-718.

Johnson, G. R. A., and Scholes, G. (Eds.). 1967. The Chemistry of Ionization and Excitation. Taylor and Francis, Ltd., London.

Joshi, D. S., Deys, B. F., Kipp, J. B. A., Barendsen, G. W., and Kralendonk. 1977. Comparison of three mammalian cell lines with respect to their sensitivities to hyperthermia, γ-rays, and U.V. radiation. Int. J. Rad. Biol. 31(5):485-492.

Kahler, H., Chalkey, H. W., and Voegtlin, C. 1929. The nature of the effect of a high frequency electric field upon paramecium. Public Health Report 44, p. 339.

Kalant, H. 1959. Physiologic hazards of microwave radiation, survey of published literature. Can. Med. Assn. J. 81:575-582.

Kaye, G. W. C., and Laby, T. H. 1973. Tables of Physical and Chemical Constants, and Some Mathematical Functions. Longman Group Limited, London.

Krusen, F. H., Herrick, J. F., Leden, U., and Wakim, K. G. 1947. Microkymatotherapy: Preliminary report of experimental studies of heating effects of microwaves (radar) in living tissues. Proc. Staff Meet. Mayo Clinic 22:209.

Kuppermann, A. 1967. Diffusion model of the radiation chemistry of aqueous solutions. In: Proceedings of the Third International Congress of Radiation Research, G. Silini, Ed. North-Holland Publishing, Amsterdam, pp. 212-234.

Latarjet, R. 1972. Interaction of radiation energy with nucleic acids. In: Current Topics in Radiation Research, Vol. 8, M. Ebert and A. Howard, Eds. North-Holland Publishing, Amsterdam, pp. 1-38.

Lea, D. E. 1946. Actions of Radiation on Living Cells. Cambridge University Press, London, 2nd Ed., 1956.

Lehmann, J. F. 1976. Diathermy. In: Handbook of Physical Medicine and Rehabilitation, Krusen, Kottke and Ellwood, Eds. Saunders, Philadelphia, Pa.

LeVeen, H., Wapnick, S., Piccone, V., Falk, G., and Ahmed, N. 1976. Tumor eradication by RF. JAMA 235(20):2198.

Lillicrap, S. C., and Fielden, E. M. 1972. Energy transfer between protein and DNA in nucleoprotein. Int. J. Radiat. Biol. 21:137.

Ling, G. N. 1971. Hydration of macromolecules. In: Water and Aqueous Solutions: Structure, Thermodynamics, and Transfer Processes, R. A. Horn, Ed. Wiley Interscience, New York.

Lorain, P., and Corson, D. 1970. Electromagnetic Fields and Waves. W. H. Freeman and Company, San Francisco.

Lords, J. L., Durney, C. H., Borg, A. M., and Tinney, C. E. 1973. Rate effects in isolated hearts induced by microwave irradiation. Proc. IEEE MTT-21:834-836.

Luse, R. A. 1964. Basic mechanisms in radiation chemistry of proteins and nucleic acids. Radiat. Res. Suppl. 4:192.

McAfee, R. D. 1961. Neurophysiological effect of 3-cm microwave radiation. Amer. J. Physiol. 200:192-194.

McAfee, R. D. 1962. Physiological effects of thermode and microwave stimulation of peripheral nerves. Amer. J. Physiol. 203:374-378.

McAfee, R. D. 1970. The neural and hormonal response to microwave stimulation of peripheral nerves. In: Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings, S. F. Cleary, Ed., pp. 150-153.

MacGregor, R. J. 1970. A direct mechanism for the influence of microwave radiation on neuroelectric potentials. Rand Corporation Publication, p. 4398.

Marha, K., Musil, J., and Tuha, H. 1971. Electromagnetic Fields and the Life Environment. San Francisco Press, Inc., San Francisco.

Mendecki, J., Friedenthal, E., Botstein, C., Sterzer, F., Paglione, R., Nowogrodski, M., and Beck, E. 1978. Microwave induced hyperthermia in cancer treatment: Apparatus and preliminary results. Int. J. Rad. Oncol. Biol. Phys. 4:1095-1103.

Michaelson, S. M. 1970. Biological effects of microwave exposure. In: Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings USDHEW, Rep. PHS, BRH, DBE 70-2, June 1970, pp. 35-58.

Michaelson, S. M. 1971. Biomedical aspects of microwave exposure. Amer. Ind. Hyg. Assn. J. 32:338-345.

Michaelson, S. M. 1972. Human exposure to non-ionizing radiant energy--potential hazards and safety standards. Proc. IEEE 60(4): 389.

Michaelson, S. M. 1974. Thermal effects of single and repeated exposures to microwaves. In: Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Proceeding of an International Symposium, Warsaw, 15-18 October 1973. Polish Medical Publishers, pp. 1-14.

Michaelson, S. M., and Dodge, C. H. 1971. Soviet views on the biological effects of microwaves--An analysis. Health Physics 21(7):108-111.

Michaelson, S. M., Thomson, R. A. E., and Howland, J. 1958. Biologic effects of microwave exposure. Griffiss AFB, New York, Rome Air Development Ctr., ASTIA Doc. AD 824-242, pp. 1-138.

Michaelson, S. M., Thomson, R. A., and El-Tamami, N. Y. 1964. The hematologic effects of microwave exposure. Aerospace Med. 35: 824-829.

Michaelson, S. M., Thomson, R. A. E., Tamami, M. Y. E., Seth, H. S., and Howland, J. W. 1962. The effects of microwaves on the response to ionizing radiation. Aerospace Med. 33:345.

Michaelson, S. M., Thomson, R. A. E., Odland, L. T., and Howland, J. W. 1963. The influence of microwaves on ionizing radiation exposure. Aerospace Med. 34:111.

Milroy, W. C., and Michaelson, S. M. 1971. Biological effects of microwave radiation. Health Physics 20:567-575.

Mondovi, B., Agro, A. F., Rotilio, G., Strom, R., Moricca, G., and Fanelli, A. R. 1969. The biochemical mechanism of selective heat sensitivity of cancer cells. II. Studies on nucleic acids and protein synthesis. Eur. J. Cancer 5:137-146.

Mondovi, B., Strom, R., Rotilio, G., Agro, A. F., Cavaliere, R., and Fanelli, A. R. 1969. The biochemical mechanism of selective heat sensitivity of cancer cells. I. Studies on cellular respiration. Eur. J. Cancer 5:129-136.

Moore, G. E., Sandberg, A. A., and Ulrich, K. 1966. Suspension cell culture and in vivo and in vitro chromosome constitution of mouse leukemia L1210. J. Natl. Cancer Inst. 36(3):405-421.

Mozumder, A. 1969. Charged particle tracks and their structure. In: Advances in Radiation Chemistry, Vol. I, M. Burton and J. Magee, Eds. Wiley Interscience, New York, pp. 1-102.

Mozumder, A., and Magee, J. L. 1966. A simplified approach to diffusion-controlled radical reactions in the tracks of ionizing radiation. Radiat. Res. 28:215-231.

Mozumder, A., and Magee, J. L. 1966. Model of tracks of ionizing radiation for radical reaction mechanisms. Radiat. Res. 28:203-214.

Neuder, S. M. 1978. Electromagnetic fields in biological media, Part I: Dosimetry--A primer on bioelectromagnetics. Bureau of Radiological Health, Rockville, Md.

Nyrop, J. E. 1946. A specific effect of high-frequency electric currents on biological objects. Nature 157:51.

Okada, S. 1970. Radiation Biochemistry, Vol. I, Cells. Academic Press, N.Y.

Okress, E. C. (Ed.). 1968. Microwave Power Engineering, Vol. I. Generation, Transmission, Rectification, and Vol. II. Applications. Academic Press, New York.

Overgaard, J. 1977. Effect of hyperthermia on malignant cells in vivo--A review and a hypothesis. Cancer 39:2637-2646.

Overgaard, K., and Overgaard, J. 1972. Investigations on the possibility of a thermic tumour therapy. II. Action of combined heat-roentgen treatment on a transplanted mouse mammary carcinoma. Eur. J. Cancer 8:63-78.

Panovsky, W. K. H., and Phillips, M. 1955. Classical Electricity and Magnetism. Addison-Wesley Publishing Company, Inc., Reading, Mass.

Paul, J. 1965. Carbohydrate and energy metabolism. In: Cells and Tissues in Culture, Vol. I, E. N. Willmer, Ed. Academic Press, N.Y., p. 239.

Paul, J. 1975. Cell and Tissue Culture, 5th Ed. E & S Livingstone Ltd., Edinborough.

Pereira, F. A. 1935. On the effect of electromagnetic waves on enzyme systems. Biochem. Z. 238:53-58.

Phillips, R. A., and Tolmach, L. J. 1966. Repair of potentially lethal damage in X-irradiated HeLa cells. Radiat. Res. 29:413.

Platzman, R. L. 1967. Energy spectrum of primary activations in the action of ionizing radiation. In: Proceedings of the Third International Congress of Radiation Research, G. Silini, Ed. North-Holland Publishing, Amsterdam, pp. 20-42.

Powers, E. R., and Tallentire, A. 1968. The roles of water in the cellular effects of ionizing radiations. In: Actions Chimiques et Biologiques des Radiations, Vol. 12, M. Haissinski, Ed. Masson et Cie., Paris, p. 3.

Prasad, K. N. 1974. Human Radiation Biology. Harper and Row, New York.

Prausnitz, S., Susskind, C., and Vogelhut, P. O. 1961. Longevity and cellular studies with microwaves. In: Proceedings of the 4th Annual Tri-Service Conference on Biological Effects of Microwave Radiating Equipment: Biologic Effects of Microwave Radiations, M. F. Peyton, Ed. Plenum Press, New York, p. 135.

Presman, A. S. 1965. The action of microwaves on living organisms and biological structures. Usp. Fiz. Nauk 86:263-302 (translated), Joint Publications Research Service (JPRS 33045), Washington, D.C.

Presman, A. S. 1970. Electromagnetic Fields and Life. Plenum Press, New York.

Puck, T. T., and Marcus, P. I. 1956. Action of X-rays on mammalian cells. J. Exp. Med. 103:653-666.

Roth, E. M. 1968. Microwave radiation. In: Compendium of Human Responses to the Aerospace Environment, E. M. Roth, Ed., Vol. 1, Sec. 1, NASA Rep. CR-1205(1).

Saito, M., and Schwan, H. P. 1961. The time constants of pearl-chain formation. In: Biological Effects of Microwave Radiation, M. F. Peyton, Ed. Plenum Press, New York, pp. 85-97.

Saito, M., Schwan, H. P., and Schwarz, G. 1966. Response of non-spherical biological particles to alternating electric fields. Biophys. J. 6:313-327.

Sawada, S., and Okada, S. 1970. Rejoining of single-strand breaks of DNA in cultured mammalian cells. Radiat. Res. 41:145.

Scholes, G. 1968. Radiolysis of nucleic acids and their components in aqueous solution. In: Radiation Chemistry of Aqueous Systems, G. Stein, Ed. Weismann Science Press, Jerusalem, p. 259.

Scholes, G., Ward, J. F., and Weiss, J. J. 1960. Mechanism of the radiation-induced degradation of nucleic acids. J. Mol. Biol. 2:379-391.

Schwan, H. 1948. Electrical properties of living tissues. Am. J. Med. Sci. 215:233.

Schwan, H. 1957. Electrical properties of tissue and cell suspensions. Adv. Biol. Med. Phys. 5:147.

Schwan, H. 1958. Molecular response characteristics to ultra-high frequency fields. In: Proceedings of the 2nd Tri-Service Conference on Biological Effects of Microwave Radiation, Rome, p. 33.

Schwan, H. P. 1959. Alternating current spectroscopy of biological substances. Proc. IRE 47:1841-1855.

Schwan, H. P. 1965. Biophysics of diathermy. In: Therapeutic Heat and Cold, S. Licht, Ed. New Haven, Conn., Sec. 3, pp. 63-125.

Schwan, H. P. 1968. Microwave biophysics. In: Microwave Power Engineering, Vol. II. Applications, E. C. Okress, Ed. Academic Press, New York, p. 213.

Schwan, H. P. 1970. Interaction of microwave and radio frequency radiation with biological systems. In: Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings, USDHEW, Rep. PHS, BRH, DBE 70-2, pp. 13-20.

Schwan, H. P. 1974. Principles of interaction of microwave fields at the cellular level. In: Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Proceedings of an International Symposium, Warsaw, 15-18 October 1973. Polish Medical Publishers, pp. 152-159.

Schwan, H. P., Anne, A., and Sher, L. 1966. Heating of living tissues. U.S. Naval Air Engineering Ctr., Philadelphia, Pa., Rep. NAEC-ACEL-534.

Schwan, H., and Piersol, G. 1954. Special review: The absorption of electromagnetic energy in body tissues. Part I. Biophysical aspects. Am. J. Phys. Med. 33:371.

Schwan, H., and Piersol, G. 1955. The absorption of electromagnetic energy in body tissues. A review and critical analysis. Part II. Physiological and clinical aspects. Am. J. Phys. Med. 34:425.

Schwiebert, M. C., and Daniels, M. 1971. ^{60}Co gamma-ray induced peroxidation of DNA in aqueous solution. Int. J. Rad. Phys. Chem. 3: 353.

Seaman, R. 1973. A summary of the ERMAC work session on nervous system and behavioral effects of nonionizing electromagnetic radiation. J. Microwave Power 10(2):127-140.

Setlow, R. B., and Pollard, E. C. 1964. Molecular Biophysics. Addison-Wesley Publishing Co., Inc., Reading, Mass.

Setlow, R. B., and Setlow, J. K. 1972. Effects of radiation on polynucleotides. Ann. Rev. Biophys. Bioeng. 1:293-346.

Sher, L. D. 1970. Interaction of microwave and RF energy on biological material. In: Electronic Product Radiation and the Health Physicist. Tech. Rep. USDHEW, PHS, BRH, DBE 70-26, pp. 431-462, Oct. 1970.

Sinclair, W. K. 1968. Cyclic X-ray response in mammalian cells in vitro. Radiat. Res. 33:620.

Sinclair, W. K. 1969. Protection by cysteamine against lethal X-ray damage during the cell cycle of Chinese hamster cells. Radiat. Res. 39:185.

Sinclair, W. K. 1969. X-ray survival of mammalian cells in culture. In: Comparative Cellular and Species Radiosensitivity, V. P. Bond and T. Sugahara, Eds. Igaku Shoin, Ltd., Tokyo, pp. 65-78.

Shadowitz, A. 1975. The Electromagnetic Field. McGraw-Hill, Inc., New York.

Shnol', S. E. 1967. Conformational vibrations of molecules. In: Vibrational Processes in Biological and Chemical Systems. Nauka, Moscow.

Shnol', S. E. 1965. Synchronous conformational vibrations of molecules of actin, myosin, and actomyosin in solutions. In: Molecular Biophysics. Nauka, Moscow, p. 56.

Sommer, H. C., and von Gierke, H. E. 1964. Hearing sensations in electrical fields. Aerospace Med. 35:834-839.

Spinks, J. W. T., and Woods, R. J. 1976. An Introduction to Radiation Chemistry. John Wiley & Sons, Inc., N.Y.

Stein, G. 1968. Excitation and ionization by ionizing radiation in water and aqueous solutions. In: Radiation Chemistry of Aqueous Systems, G. Stein, Ed. Weizmann Science Press, Jerusalem, pp. 83-89.

Stodolnik-Baranska, W. 1967. Lymphoblastoid transformation of lymphocytes in vitro after microwave irradiation. Nature 214:102-103.

Stodolnik-Baranska, W. 1974. The effects of microwaves on human lymphocyte cultures. In: Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski et al., Eds. Proceedings of an International Symposium, Warsaw, 15-18 October 1973. Polish Medical Publishers, pp. 189-195.

Streffer, C., von Buenîngen, D., Dietzel, F., Rottinger, E., Robinson, J. E., Sherer, E., and Trott, R. B. 1978. Cancer Therapy by Hyperthermia and Radiation, Proceedings of the Second International Symposium, Essen, Germany, June 2-4, 1977. Urban and Schwarzenberg, Baltimore, Munich.

Stuchly, S. S., and Hamid, M. A. K. 1972. Physical parameters in microwave heating. J. Microwave Power 7(2):117-137.

Sybenga, J. 1972. General Cytogenetics. American Elsevier Publishing Co., Inc., New York.

Takashima, Shiro. 1963. Dielectric dispersion of DNA. J. Mol. Biol. 7:455-467.

Takashima, Shiro. 1966. Studies on the effect of radiofrequency waves on biological macromolecules. IEEE Trans. Biomedical Engineering BME-13(1):28-31.

Teixeira-Pinto, A. A., Nejelski, L. L., Cutler, J. L., and Heller, J. H. 1960. The behavior of unicellular organisms in an electromagnetic field. Exp. Cell Res. 20:548-564.

Thomas, J. K. 1969. Elementary processes and reactions in the radiolysis of water. In: Advances in Radiation Chemistry, Vol. I, M. Burton and J. Magee, Eds. Wiley Interscience, New York, pp. 103-198.

Thrall, D. E., Gerweck, L. E., Gillette, E. L., and Dewey, W. C. 1976. Response of cells in vitro and tissues in vivo to hyperthermia and x-irradiation. In: Advances in Radiation Biology, Vol. 6. Academic Press Inc., New York, pp. 211-227.

Ullrich, M., and Hagan, U. 1971. Base liberation and concomitant reactions in irradiated DNA solutions. Int. J. Radiat. Biol. 19(6): 507-517.

Van Ummersen, C. 1961. The effect of 2450 Mc radiation on the development of the chick embryo. In: Biological Effects of Microwave Radiation, Vol. 1, M. F. Peyton, Ed. Plenum Press, New York, p. 201.

Valtonen, E. J. 1966. Giant mast cells - a special degenerative form produced by microwave radiation. Exp. Cell Res. 43:221.

Vogelhut, P. 1960. Study of enzymatic activity under the influence of 3-cm electromagnetic radiation. Third International Conference on Medical Electronics, p. 52.

Vogelhut, P. O. 1968. Microwave techniques in biophysical measurements. J. Microwave Power 3(3):143-147.

Vogelman, J. H. 1958. Physical characteristics of microwaves as related to biological effects. In: Proceedings of the 2nd Annual Tri-Service Conference on Biological Effects of Microwave Energy. Springfield, Va. Clearing House, Dep. of Commerce, ASTIA Doc. AD 131477, pp. 9-18.

Vogelman, J. H. 1970. Physical characteristics of microwave and other radio frequency radiation. In: Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings, S. F. Cleary, Ed. USDHEW, Rep. PHS, BRH, DBE 70-2, June, 1970, pp. 7-12.

Walker, C. M. B., McWhirter, K. G., and Voss, W. A. G. 1974. Use of a bacteriophage system for investigating the biological effects of low intensity pulsed microwave radiation. J. Microwave Power 9(3): 221-229.

Watanabe, I., and Okada, S. 1966. Study of mechanisms of radiation-induced reproductive death of mammalian cells in culture: Estimation of stage at cell death and biological description of processes leading to cell death. Radiat. Res. 27:290.

Watanabe, I., and Okada, S. 1967. Effects of temperature on growth rate of L5178Y cells. J. Cell Biol. 32:309.

Weiss, J. J. 1967. Electron transfer processes in radiation induced reactions. In: The Chemistry of Ionization and Excitation, G. R. A. Johnson and G. Scholes, Eds. Taylor and Francis, Ltd., London, pp. 17-23.

Weiss, B. G. 1971. Perturbation in precursor incorporation into DNA of X-irradiated HeLa cells. Radiat. Res. 48:128.

Westra, A., and Dewey, W. C. 1971. Variations in sensitivity to heat shock during the cell cycle of Chinese hamster cells in vitro. Int. J. Radiat. Biol. 19:467.

Whitmore, G. F., Stanners, C. P., Till, J. E., and Gulyas, S. 1961. Nucleic acid synthesis and the division cycle in X-irradiated L-strain mouse cells. Acta Biochem. Biophys. 47:66-77.

Willmer, E. N. 1965. Morphological problems of cell type, shape, and identification. In: Cells and Tissues in Culture, Vol. I, E. N. Willmer, Ed. Academic Press, N.Y., p. 143.

Winans, L. F., Dewey, W. C., and Dettor, C. M. 1972. Repair of sub-lethal and potentially-lethal damage in synchronous Chinese hamster cells. Radiat. Res. 52:333.

Yeargers, E. K., Langley, J. B., Sheppard, A. P., and Huddleston, G. K. 1975. Effects of microwave radiation on enzymes. In: Biologic Effects of Nonionizing Radiation, D. Tyler, Ed. Ann. N.Y. Acad. Sci. 247:301-304.

APPENDIX I

Calculation of mean generation time (MGT) using logarithms to base 2.

When a cell population increases in number from N_1 to N_2, the difference between the logarithms to the base 2 of N_1 and N_2 is the number of doubling that occurred during the time interval $t_2 - t_1$.

Example: At t = 0 hours, the cell number = 0.5×10^5 cells/ml

At t = 24 hours, the cell number = 0.183×10^6 cells/ml

$$\log_2 0.5 \times 10^5 = \log_2 500 + \log_2 100$$
$$= 8.966 + 6.644$$
$$= 15.610$$

$$\log_2 0.183 \times 10^6 = \log_2 183 + \log_2 10^3$$
$$= 7.516 + 9.966$$
$$= 17.482$$

$$\log_2 N_2 - \log_2 N_1 = \text{number of doublings}$$

$$17.482 - 15.610 = 1.872$$

$$\text{MGT} = \frac{24 \text{ hours}}{1.872 \text{ doublings}} = 12.8 \text{ hours}$$

LOGARITHMS TO BASE 2

(Finney, Hazlewood, and Smith, 1955)

	0	1	2	3	4	5	6	7	8	9
100	6.644	6.658	6.672	6.687	6.700	6.714	6.728	6.741	6.755	6.768
110	.781	.794	.807	.820	.833	.845	.858	.870	.883	.895
120	.907	.919	.931	.943	.954	.966	.977	.989	7.000	7.011
130	7.022	7.033	7.044	7.055	7.066	7.077	7.087	7.098	.109	.119
140	129	.140	.150	.160	.170	.180	.190	.200	.209	.219
150	7.229	7.238	7.248	7.257	7.267	7.276	7.285	7.295	7.304	7.313
160	.322	.331	.340	.349	.358	.366	.375	.384	.392	.401
170	.409	.418	.426	.435	.443	.451	.459	.468	.476	.484
180	.492	.500	.508	.516	.524	.531	.539	.547	.555	.562
190	.570	.577	.585	.592	.600	.607	.615	.622	.629	.637
200	7.644	7.651	7.658	7.665	7.672	7.679	7.687	7.693	7.700	7.707
210	.714	.721	.728	.735	.741	.748	.755	.762	.768	.775
220	.781	.788	.794	.801	.807	.814	.820	.827	.833	.839
230	.845	.852	.858	.864	.870	.877	.883	.889	.895	.901
240	.907	.913	.919	.925	.931	.937	.943	.948	.954	.960
250	7.966	7.972	7.977	7.983	7.989	7.994	8.000	8.006	8.011	8.017
260	8.022	8.028	8.033	8.039	8.044	8.050	.055	.061	.066	.071
270	.077	.082	.087	.093	.098	.103	.109	.114	.119	.124
280	.129	.134	.140	.145	.150	.155	.160	.165	.170	.175
290	.180	.185	.190	.195	.200	.205	.209	.214	.219	.224
300	8.229	8.234	8.238	8.243	8.248	8.253	8.257	8.262	8.267	8.271
310	.276	.281	.285	.290	.295	.299	.304	.308	.313	.317
320	.322	.326	.331	.335	.340	.344	.349	.353	.358	.362
330	.366	.371	.375	.379	.384	.388	.392	.397	.401	.405
340	.409	.414	.418	.422	.426	.430	.435	.439	.443	.447
350	8.451	8.455	8.459	8.464	8.468	8.472	8.476	8.480	8.484	8.488
360	.492	.496	.500	.504	.508	.512	.516	.520	.524	.527
370	.531	.535	.539	.543	.547	.551	.555	.558	.562	.566
380	.570	.574	.577	.581	.585	.589	.592	.596	.600	.604
390	.607	.611	.615	.618	.622	.626	.629	.633	.637	.640
400	8.644	8.647	8.651	8.655	8.658	8.662	8.665	8.669	8.672	8.676
410	.679	.683	.687	.690	.693	.697	.700	.704	.707	.711
420	.714	.718	.721	.725	.728	.731	.735	.738	.741	.745
430	.748	.752	.755	.758	.762	.765	.768	.771	.775	.778
440	.781	.785	.788	.791	.794	.798	.801	.804	.807	.811
450	8.814	8.817	8.820	8.823	8.827	8.830	8.833	8.836	8.839	8.842
460	.845	.849	.852	.855	.858	.861	.864	.867	.870	.873
470	.877	.880	.883	.886	.889	.892	.895	.898	.901	.904
480	.907	.910	.913	.916	.919	.922	.925	.928	.931	.934
490	.937	.940	.943	.945	.948	.951	.954	.957	.960	.963
500	8.966	8.969	8.972	8.974	8.977	8.980	8.983	8.986	8.989	8.992
510	.994	.997	9.000	9.003	9.006	9.008	9.011	9.014	9.017	9.020
520	9.022	9.025	.028	.031	.033	.036	.039	.042	.044	.047
530	.050	.053	.055	.058	.061	.063	.066	.069	.071	.074
540	.077	.079	.082	.085	.087	.090	.093	.095	.098	.101

	0	1	2	3	4	5	6	7	8	9
550	9.103	9.106	9.109	9.111	9.114	9.116	9.119	9.122	9.124	9.127
560	.129	.132	.134	.137	.140	.142	.145	.147	.150	.152
570	.155	.157	.160	.162	.165	.167	.170	.172	.175	.177
580	.180	.182	.185	.187	.190	.192	.195	.197	.200	.202
590	.205	.207	.210	.212	.214	.217	.219	.222	.224	.226
600	9.229	9.231	9.234	9.236	9.238	9.241	9.243	9.246	9.248	9.250
610	.253	.255	.257	.260	.262	.264	.267	.269	.271	.274
620	.276	.278	.281	.283	.285	.288	.290	.292	.295	.297
630	.299	.301	.304	.306	.308	.311	.313	.315	.317	.320
640	.322	.324	.326	.329	.331	.333	.335	.338	.340	.342
650	9.344	9.347	9.349	9.351	9.353	9.355	9.358	9.360	9.362	9.364
660	.366	.369	.371	.373	.375	.377	.379	.382	.384	.386
670	.388	.390	.392	.394	.397	.399	.401	.403	.405	.407
680	.409	.412	.414	.416	.418	.420	.422	.424	.426	.428
690	.430	.433	.435	.437	.439	.441	.443	.445	.447	.449
700	9.451	9.453	9.455	9.457	9.459	9.461	9.464	9.466	9.468	9.470
710	.472	.474	.476	.478	.480	.482	.484	.486	.488	.490
720	.492	.494	.496	.498	.500	.502	.504	.506	.508	.510
730	.512	.514	.516	.518	.520	.522	.524	.526	.527	.529
740	.531	.533	.535	.537	.539	.541	.543	.545	.547	.549
750	9.551	9.553	9.555	9.557	9.558	9.560	9.562	9.564	9.566	9.568
760	.570	.572	.574	.576	.577	.579	.581	.583	.585	.587
770	.589	.591	.592	.594	.596	.598	.600	.602	.604	.605
780	.607	.609	.611	.613	.615	.617	.618	.620	.622	.624
790	.626	.628	.629	.631	.633	.635	.637	.638	.640	.642
800	9.644	9.646	9.647	9.649	9.651	9.653	9.655	9.656	9.658	9.660
810	.662	.664	.665	.667	.669	.671	.672	.674	.676	.678
820	.679	.681	.683	.685	.687	.688	.690	.692	.693	.695
830	.697	.699	.700	.702	.704	.706	.707	.709	.711	.713
840	.714	.716	.718	.719	.721	.723	.725	.726	.728	.730
850	9.731	9.733	9.735	9.736	9.738	9.740	9.741	9.743	9.745	9.747
860	.748	.750	.752	.753	.755	.757	.758	.760	.762	.763
870	.765	.767	.768	.770	.771	.773	.775	.776	.778	.780
880	.781	.783	.785	.786	.788	.790	.791	.793	.794	.796
890	.798	.799	.801	.803	.804	.806	.807	.809	.811	.812
900	9.814	9.815	9.817	9.819	9.820	9.822	9.823	9.825	9.827	9.828
910	.830	.831	.833	.834	.836	.838	.839	.841	.842	.844
920	.845	.847	.849	.850	.852	.853	.855	.856	.858	.860
930	.861	.863	.864	.866	.867	.869	.870	.872	.873	.875
940	.877	.878	.880	.881	.883	.884	.886	.887	.889	.890
950	9.892	9.893	9.895	9.896	9.898	9.899	9.901	9.902	9.904	9.905
960	.907	.908	.910	.911	.913	.914	.916	.917	.919	.920
970	.922	.923	.925	.926	.928	.929	.931	.932	.934	.935
980	.937	.938	.940	.941	.943	.944	.945	.947	.948	.950
990	.951	.953	.954	.956	.957	.959	.960	.961	.963	.964
	10	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}
	3.322	6.644	9.966	13.288	16.610	19.932	23.253	26.575	29.897	33.219